MICROBIAL COMMUNITY DYNAMICS IN LONG-TERM NO-TILL AND CONVENTIONALLY TILLED SOILS OF THE CANADIAN PRAIRIES

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ABSTRACT

Adoption of no-till (NT) and reduced tillage management is widespread on the Canadian prairies and together form the basic platform of soil management upon which most crop production is based. Elimination of tillage in cropping systems changes the physical and chemical characteristics of the soil profile and can affect crop growth and ultimately yield. As such, understanding how soil biota, as drivers of nutrient turnover, adapt to NT is important for maximizing crop productivity and mitigating environmental damage in agroecosystems. This work aims to achieve a greater understanding of microbial community structure and function in long-term NT versus conventionally tilled (CT) soils. Community phospholipid and DNA fingerprinting did not reveal any consistent tillage-induced shifts in microbial community structure, but demonstrated a clear influence of depth within the soil profile. While tillage did not result in broad changes in the community structure, total, bacterial and fungal biomass was consistently greater near the surface of NT soils. Further examination at one site near Swift Current, SK revealed differences in microbial biomass and community structure in NT and CT in field-formed aggregate size fractions. Measurement of mineralization and nitrification at the same site indicated that differences in the early-season turnover of N may be related to physical rather than microbial differences in NT and CT soils. Potential nitrification was higher prior to seeding than mid-season, was not affected by tillage and was correlated with ammonia oxidizer population size of archaea, but not bacteria. This work indicates that edaphic soil properties and spatial distribution of resources in the soil profile, rather than tillage management, are the primary factors driving microbial community structure in these soils.

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LIST OF ABBREVIATIONS

AMF	arbuscular mycorrhizal fungi
ANOVA	analysis of variance
AOA	ammonia oxidizing bacteria
AOB	ammonia oxidizing archaea
CEC	cation exchange capacity
CT	conventional tillage
DGGE	denaturing gradient gel electrophoresis
FAME	fatty acid methyl ester
F:B	fungal:bacterial biomass ratio
GM	gross mineralization
GN	gross nitrification
GRSP	glomalin-related soil protein
Gr+	gram positive bacteria
Gr-	gram negative bacteria
MDS	non-metric multidimensional scaling
MRPP	multiple response permutation procedure
NT	no-till
PCR	polymerase chain reaction
PLFA	phospholipid fatty acid analysis
PN	potential nitrification
RCBD	randomized complete block design
RT	reduced tillage
SOM	soil organic matter
SDZ	sulfadiazine
TC	total C
TN	total N
T-RFLP	terminal restriction fragment length polymorphism
WSA	water stable aggregate

1.0 INTRODUCTION

No-till (NT) is an agricultural management practice in which the only soil disturbance event occurs at planting. Typically, about 25 to 30% of the soil surface is disturbed and seeding is achieved using specially designed openers which create a narrow band in which seed (and in some cases fertilizer) can be placed. No-till management has economic and environmental benefits and is becoming increasingly popular in commercial crop production systems. By contrast, conventional tillage (CT) management involves more rigorous physical disturbance of the soil, incorporating much of the standing crop residue. The intensity of tillage performed in CT systems typically is regionally defined, dependent on soil and climatic conditions. In CT systems, tillage is used for weed control, crop residue management and seedbed preparation. Reduced tillage (RT) aims to minimize soil disturbance but involves either occasional tillage or other light soil disturbance to optimize soil conditions for crop growth.

Currently, approximately 45% of seeded agricultural land in Canada (ca. 13.4 million hectares) is under NT management with adoption of NT exceeding 50% in the Prairie provinces (Statistics Canada, 2008). Further, more than 70% of cropland in Canada is managed using some form of RT. This widespread conversion from CT to NT occurred over a short timeframe, with limited adoption prior to 1990. In the Prairie region of Canada, NT and RT soils are becoming the basic platform on which food production systems are based and understanding the impact of NT in the myriad of diverse cropping systems in western Canada remains an ongoing endeavor.

Given the potential economic and environmental impacts of such a profound change in soil management, significant research has been conducted in NT systems. Questions of immediate consequence, including the influence of NT on agronomic parameters such as crop yield and quality, have been extensively addressed. As our knowledge of these production

implications grows, additional focus has been directed toward elucidating longer-term impacts of NT on agroecosystem function. In particular, focus on increased soil C storage as an offset to greenhouse gas emissions in some soil systems has brought to light the significance and utility of a longer-term perspective.

Considerable impacts of NT on soil biota, including both microorganisms and soil fauna, have been well documented (Kladivko, 2001). For example, abundance of earthworms is greater in NT than CT systems (Wardle, 1995). The importance of the mixing action provided by earthworm populations is clear in NT soil, where mechanical mixing is minimal. Similarly, other litter decomposers may have a more important role in NT soils than in soils where residue soil contact is facilitated by tillage events. Studies in the US Great Plains indicated that under RT and NT cropping systems there was a shift in the microbial decomposer community towards one dominated by fungi, particularly at the soil surface (Beare et al., 1997; Frey et al., 1999; Guggenberger et al., 1999). This increased fungal biomass, attributed to decreased physical disturbance and heterogeneity of spatial resource distribution in NT systems, is offered as a mechanism affecting C storage in aggregates (Beare et al., 1997; Guggenberger et al., 1999; Simpson et al., 2004) and N translocation and availability during residue decomposition (Frey et al., 2000).

While the microbial biomass in NT systems has been examined in a number of US soils, little work has been done in Canada. Perhaps more importantly, much of the previous work has included only biomass measurements and has not examined the genetic and functional diversity of the microbial community. Increased microbial biomass in NT has the potential to affect the species composition of the resulting communities by changing availability and forms of nutrients. Macro- and microaggregate stability may also be affected by changes in microbial

community dynamics, especially where fungi have a more prominent role in litter decomposition. Macroaggregates are important for soil structure, air and water movement. Microaggregates protect soil organic matter in the long term (Six et al., 2004a) and are of particular interest as bacterial diversity is greater in small rather than coarse size fractions (Sessitch et al., 2001). Reduced macroaggregate turnover in NT soils may impact the diversity of indigenous bacterial and fungal communities.

Nitrogen availability in NT systems can be markedly different than under CT management, often resulting in lower grain yield and protein content (McConkey et al., 2002). Reduced N availability has been attributed to lower N mineralization during the growing season (McConkey et al., 2002), immobilization of N in the litter layer (Frey et al., 2000) and higher gaseous losses from denitrification (N_2, N_2O) (Aulakh et al., 1984). Microscale microbial community dynamics and transformations of N in NT soils are largely unstudied, and the relative contribution of fungi and bacteria to N cycling has not been examined. Although fungal deposition of C in microaggregates within macroaggregates is an important means by which C can be stored in NT soils (Sessitch et al., 2001), turnover of N in aggregates and the microbes responsible are not well understood. Increased influence of fungi on residue decomposition and N-cycling may substantially affect crop N availability and susceptibility to nutrient loading, influencing various N losses into adjacent ecosystems. For example, recent work indicates that fungal denitrification can result in significant production of N_2O (Laughlin and Stevens, 2002) and emissions may be of ecological importance in soils where fungal activity is dominant.

Physiological redundancy in soil microbial communities is well documented but a shift in bacterial-fungal dynamics may have a significant effect on numerous indispensable soil functions other than nutrient cycling, including contributions to water quality and contaminant

degradation (e.g., pesticides). Given the ever-increasing prevalence of NT management in Canada, changes in the diversity of the fungal and bacterial communities in NT agricultural soils is of significant interest and importance for the sustainability of Canadian agroecosystems.

2.0 LITERATURE REVIEW

2.1 History of no-till on the Canadian prairies

Recognition of the degradation of soil resources by monocropping and summerfallow was documented as early as 1910. Janzen (2001) cites that Angus McKay, superintendent at Indian Head Experimental Farm, observed in 1889 that allowing crop residue to remain on the soil surface until spring (as opposed to fall ploughing) retained snow and conserved moisture. However, the longstanding opinion at the time was that summerfallow was a successful method of moisture retention and intensive cultivation of the soil dominated for nearly a century.

Pioneers of NT management, producers and professional researchers alike, lived and worked on the Canadian Prairies and the development and adoption of NT have been touted as one of agriculture's most prominent success stories. Development of new herbicides made the practices of chemical fallow and NT possible by eliminating weed pressure normally controlled with tillage (Appleby, 2005). Affordability of NT seeding equipment and herbicides (primarily glyphosate) improved, making NT a more viable option. Severe drought on the prairies in the early 1980's further emphasized the utility of NT for conserving soil moisture and preventing soil erosion. These combined factors lead to the widespread adoption of NT in Saskatchewan and across the prairies in the 1990's. In 1987 the Canadian Soil Conservation Council of Canada was formed, and along with various provincial organizations (e.g., the Saskatchewan Soil Conservation Association, Alberta Reduced Tillage Linkages and the Manitoba Zero Tillage Research Association) they promoted an understanding and awareness of soil conservation issues. These organizations have been instrumental in the collection and dissemination of information on the benefits of NT in western Canadian crop production systems.

No-till on the prairies generally results in decreased risk of soil erosion and enhances moisture conservation as a consequence of residue retention on the soil surface. Long-term yield increases have been observed under NT (Lafond et al., 2006); however, increased crop yield does not always occur (McConkey et al., 1996). The influence of NT on crop yield often varies from year to year and is dependent on the interaction of factors such as climate, soil and crop types as well as other management practices. Further, net profitability among tillage management systems is reportedly dependent on location, with far greater benefits achieved in the Black and Gray soil zones, largely as a function of yield benefits and the elimination of substantial cost associated with frequent tillage operations (Zentner et al., 2002). Despite this apparent gradient of economic impact, adoption of RT and NT is widespread across the Canadian Prairies, occurring in all soil zones. Enhanced soil conservation and sustainability of agricultural agroecosystems under NT management will likely result in further adoption on lands currently under both CT and RT.

2.2 Changes in soil properties under no-till

Soil conditions are affected by reduced disturbance and altered residue placement under NT. In western Canada, increased storage capacity and conservation of soil moisture is often the greatest benefit for crop growth under NT, but the extent of the benefit conferred varies across soil and climatic zones. In the Brown soil zone, McConkey et al. (1996) showed that the influence of tillage on moisture conservation was dependent on soil texture and cropping frequency. For example, in a wheat-fallow system NT did not always convey an advantage, particularly when adequate soil moisture stores were built in the fallow period, while in continuous cropping systems NT was more likely to result in significant moisture benefits for crop growth. In the Black soil zone, Lafond et al. (2006) showed that an increase in soil water

content in the 0- to 30-cm depth resulted in a long-term yield benefit. A positive influence of soil moisture on crop yield also was observed by Soon and Arshad (2004) in a Gray Luvisolic soil. The benefit of increased soil water content under NT therefore appears to hold true for all climatic regions on the Prairies, despite differences among these soils in growing season moisture deficit.

Changes in soil pH under NT generally are non-significant. However, when initial soil pH is low, further acidification can occur and may result from gains in organic matter (Arshad et al., 1990). Duiker et al. (2006) found that cation exchange capacity (CEC) in the 0- to 15-cm layer was not affected by tillage. However a vertical stratification of CEC occurred under NT and resulted in a significantly higher CEC in the 0- to 5-cm depth.

Soil bulk density under NT is generally lower at the soil surface (0 to 5 cm) but is greater in the remainder of the plough layer (ca. 5 to 20 cm) when compared to CT (Kay and VandenBygaart, 2002). Accumulation of organic matter drives reductions in bulk density at the soil surface under NT. Whereas total porosity (i.e., bulk density) is lower in NT than CT, a significant shift in pore size distribution occurs resulting in larger average pore size (Young and Ritz, 2000; Kay and VandenBygaart, 2002). Macropores exert a large influence on soil aeration as water flows primarily through these channels during infiltration and drainage. Larger, more continuous pore systems may increase the risk of nutrient loss, especially NO_3^- in drainage and groundwater (Izaurralde et al., 1995). However, better soil structure at the surface under NT can reduce the risk of nutrient loss to adjacent environments through runoff.

Soil C storage in NT has been the subject of intense study in recent years due to the potential to use NT as a greenhouse gas mitigation strategy (Janzen et al., 1998; Lal et al, 1999; Paustian et al., 2000). Despite intense scrutiny and many recent advances, we still have much to

learn about C dynamics in tillage systems. Fully examined, this subject could provide fodder for an entire dissertation. The following provides a very brief summary of relevant information and will be supplemented during a discussion of aggregation and C dynamics in a later section of this review.

Organic C content of soil is a function of the rate of C input and decomposition rate, both of which can be affected by tillage management (Peterson et al., 1998). Decomposition of C can be directly affected by tillage management due to altered residue distribution as well as indirectly through changes in soil moisture and temperature, which occur in some regions. The effect of tillage on C sequestration is a function of both residue quantity and quality (Wright and Hons, 2005) and can also be greatly dependent on edaphic factors, especially soil texture (Campbell et al., 1996a; Campbell et al., 1996b; Chivenge et al., 2007). Stabilisation of organic C under NT can occur as the result of physical occlusion of particulate organic matter in aggregates (Tisdall and Oades, 1982; Elliott, 1986; Six et al., 2000) as well as through enhanced deposition of biochemically recalcitrant microbial residues (Chantingy et al., 1997; Guggenberger et al., 1999).

An important consideration regarding the use of reduced tillage for C sequestration is accounting for the net C storage within the soil profile. The increased rate of C accumulation under NT occurs primarily at the soil surface, whereas in CT substantial C can be accrued at depth, especially in humid climates (Angers et al., 1997; VandenBygaart et al., 2002; Olchin et al., 2008; Poirier et al., 2009). A review of agricultural management effects on SOC showed that Chernozemic soils on the Canadian prairies have a greater ability to store C under NT than other agricultural soils in Canada, especially those in the sub-humid Black soil zone (VandenBygaart et al., 2003).

Modified soil C content and especially heterogeneity of C (both form and spatial distribution) under NT, combined with edaphic factors, have enormous potential to influence the soil microbial community. Whether the changes induced under NT result in a shift in the overall abundance of microbial biomass, a shift in community structure or most importantly a change in overall function remains uncertain.

2.3 Influence of tillage on microbial communities

2.3.1 Microbial biomass and abundance

Most soil organisms, including both macro and microfauna, are more abundant under NT than CT (Wardle et al., 1995; Kladivko, 2001). Increases in microbial biomass C (Chantigny et al., 1997; Lupwayi et al., 2004) and N (Soon and Arshad, 2004; Spedding et al., 2004) under NT are generally greatest near the soil surface. Microbial biomass dynamics under different tillage management have been investigated in a number of soils and cropping systems and results are highly variable with increased microbial biomass under NT in some but not all cases (Frey et al., 1999; Drijber et al., 2000; Carpenter-Boggs et al., 2003; Feng et al., 2003; Jackson et al., 2003; Spedding et al., 2004; Acosta-Martinez et al., 2007; Minoshima et al., 2007).

It is frequently postulated that CT soils are dominated by bacteria, whereas NT soils are fungally dominant (Beare, et al., 1992; Moore, 1994; Frey et al., 1999). Fungi are better adapted than bacteria to periodic stress (Dighton, 2003) which may be more frequent in NT than CT soils. Their indeterminate growth enables them to translocate nutrients through hyphal networks, often bridging air gaps (Holland and Coleman, 1987; Klein and Paschke, 2004). This allows them to better handle environments where there is large spatial heterogeneity in resource distribution. Soil fungi are more adapted to growth at low temperatures than bacteria (Pietikainen

et al., 2005) which may provide a competitive advantage in NT. In NT, increased retention of residue on the soil surface keeps the soil surface cooler than in CT systems where trash is buried. Fungal hyphae provide links between spatially separated resources and can transfer nutrients among microsites of optimal and suboptimal physicochemical conditions (Dighton, 2003). Frey et al. (2000) demonstrated the ability of fungi to translocate N from the mineral soil upward into residue on the soil surface during decomposition in NT soils, resulting in significant N immobilization.

The relative biomass of fungi and bacteria in NT vs. CT soil was investigated in six soils in the United States Great Plains. An increase in the proportion of the total microbial biomass composed of fungi relative to bacteria was observed in five of six instances (Frey et al., 1999). This shift was related to differences in soil moisture along a climatic gradient. In western Canada, the level of physical disturbance in CT systems is positively correlated with the climatic gradient along which soil moisture status increases. In other words, in drier areas, the difference in physical disturbance between conventionally tilled soils and NT is much less than in more humid regions. It is possible therefore, that an apparent correlation between the proportional increase in the fungal vs. bacterial biomass and soil moisture could in fact be directly correlated to differences in physical disturbance.

In an evaluation of fungal and bacterial dynamics in NT and CT soils in a corn cropping system in eastern Canada, Spedding et al. (2004) found that seasonal differences in microbial community dynamics were greater than those associated with tillage treatments. A lack of tillage response may be partially explained by the humid climatic conditions in the study region. Benefits of NT on microbial growth have, in some cases, been related to increased soil moisture status; however, in the humid climate of Quebec, moisture is seldom limiting. In this way, the

temporal differences in resource availability as a function of crop growth could be more important than decreased soil disturbance for microbial community dynamics.

These disparate results likely reflect the complexity of different cropping and soil management systems, each of which may have vastly different drivers of soil chemical and biological processes, despite similarities in design with respect to applied tillage treatments. Determination of the abundance of different microbial groups in soil as a function of tillage disturbance is thus difficult to assess across agroecosystems especially while keeping in mind that measures of microbial abundance do not necessarily indicate activity or function.

2.3.2 Microbial diversity and function

Tillage effects on microbial diversity have not been studied to the same extent as microbial abundance. However, it is known that microbial community dynamics are affected by resource quantity, quality and spatial distribution (Henricksen and Breland, 1999; Young and Ritz, 2000; Acosta-Martinez et al., 2007). All of these factors can be altered as a function of tillage management which seems to affect microbial function most markedly when conditions are sub-optimal for growth (e.g., in poor quality soils) (Lupwayi et al., 2001; Cookson et al., 2008). Soils with high heterogeneity of substrates (e.g., C and N) have greater microbial diversity and greater spatial isolation of resources (i.e., higher niche variation) can also increase diversity (Torsvik and Ovreås, 2000; Tiedje et al., 2001). Spatial heterogeneity of resources is one of the key defining features of NT cropping systems and therefore has the potential to increase microbial diversity (Young and Ritz, 2000).

General improvements in soil quality through increased organic matter under NT can affect microbial functioning. This can occur indirectly through influences of soil quality on plant

growth. For example, Eo and Nakamoto (2008) found that biological activity under reduced till management was stimulated by increased root growth as a function of greater soil organic matter. Similarly, Hoflich et al. (1999) found increased bacterial diversity and abundance in the rhizosphere under reduced tillage, which they attributed to plant residues in the topsoil. Alternatively, improved quality and/or availability of soil organic C and N sources utilized directly by soil organisms in NT soils can bolster microbial activity (White and Rice, 2009).

Much of our current understanding of microbial diversity in NT vs. CT soils has been derived through the use culture-dependent techniques. For example, reduced bacterial functional diversity in CT vs. NT was measured by C substrate utilization by Lupwayi et al. (1998; 2004). Their work demonstrated the importance of sampling depth in the study of NT effects on soil microbial communities. Specifically, early work showed increased microbial biomass C and functional diversity in NT vs. CT at the 0 to 7.5cm depth (Lupwayi et al., 1998). A later study in the same soil showed that when only the 0 to 5cm depth was considered, tillage affected microbial parameters; however, there was no significant difference between tillage treatments over the 0- to 20-cm depth (Lupwayi et al., 2004). Carpenter-Boggs et al. (2003) similarly found little difference between NT and CT soils (0- to 15-cm depth) for a suite of microbial activity assays, except for C mineralization and alkaline phosphatase activity which were higher in NT. Acosta-Martinez et al. (2007) showed changes in key soil enzymes activities at the soil surface (0 to 5cm) under NT in a wheat-fallow rotation.

The use of fatty acid profiling has been used in a number of studies to assess microbial community dynamics as a function of tillage. Fatty acid analysis, and most especially phospholipid fatty acid analysis (PLFA) (Drevosky et al., 2004) can provide a powerful tool for differentiating soil microbial communities, garnering the greatest amount of statistically relevant

information when compared to substrate utilization or PCR-based fingerprinting methods (Ramsay et al., 2006). Short-term effects of tillage management have been demonstrated by Calderon et al. (2001) and Jackson et al. (2003) who examined the effect of tillage within days of the soil disturbance event and by Minoshima et al. (2007) who detected a shift in the composition of the microbial community structure at the soil surface in the first year following conversion to NT. Differences between tillage treatments were demonstrated using PLFA by Feng et al. (2003); however, the impact of tillage was not significant at all times during the growing season. Spedding et al. (2004) found that seasonal and depth effects were greater than those of tillage on PLFA profiles. Drijber et al. (2000) similarly found that NT and CT communities were best differentiated during the fallow phase of the cropping rotation. Selection of sampling time and consideration of depth are important for determining the effect of tillage disturbance on microbial diversity and multiple approaches to community analysis may be required to elucidate differences in microbial communities in soil where populations may be similar in composition (Lawlor et al., 2000).

To date, the use of DNA based methods to study tillage effects on soil microbial communities have not been frequently used. Piexoto et al. (2006) studied aggregate size distribution and community structure in NT and CT soils in Brazil. Their work showed that aggregate size distribution and community structure changed as a function of tillage. Specifically, NT soils had more macroaggregates than CT soils. Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) fingerprinting demonstrated differences in bacterial communities in the bulk soils (0 to15 cm) from NT and CT soils and revealed greater similarity between NT and an adjacent forest soil than between NT and CT, as well as little effect of soil depth.

Spatial heterogeneity of resources is a fundamental property of NT soils and niche variation is thought to increase diversity (Torsvik and Ovreås, 2002). Given the small spatial scale in which microbes function, changes to soil structure which affect niche distribution may affect microbial communities to a greater extent than broad shifts in bulk soil characteristics. If shifts in aggregate distribution occur under NT, then it is possible that broader effects of NT on microbial community functioning may occur.

2.4 Effects of no-till on aggregation

Aggregates are vital for sustainable soil functioning through the provision of structural stability and good porosity. Good aggregation provides resistance to wind and water erosion as well as improving water infiltration and crop water use efficiency. Aggregates provide microhabitat for soil biota and protect and store organic matter which makes them central to sustainable soil management in agricultural production systems. Agricultural management practices which increase crop productivity and decrease soil physical disturbance serve to enhance aggregate formation and stability and contribute to good soil structure.

2.4.1 Mechanisms of aggregate formation

Tisdall and Oades (1982) first proposed a hierarchal theory of aggregate formation which specifies that binding agents of differing persistence act in a sequential manner to form hierarchical stages of aggregation. The first stage involves the binding of primary particles and silt-sized aggregates (<20µm) into microaggregates by persistent binding agents, mainly microbial mucilages and humified organic matter. This is followed by binding of stable microaggregates into macroaggregates by more transient binding agents such as fungal hyphae,

roots and polysaccharides. Their aggregate hierarchy theory has since evolved to provide additional insight into the mechanisms of aggregate formation and stabilisation. An important modification to the original theory was the recognition that aggregate formation is not exclusively a hierarchal process in that macroaggregate binding materials may serve as the starting material for the formation of microaggregates within an existing macroaggregate (Oades, 1984). It is this particular aspect of aggregate formation theory that has lead to important theories regarding the long-term protection and stabilisation of soil organic C (Six et al., 2000; 2004a).

2.4.2 Role of glomalin in aggregation

Arbuscular mycorrhizal fungi (AMF) may have an especially vital role in the formation and particularly the stabilization of aggregates. AMF are ubiquitous in agricultural soils on the Canadian prairies and AMF population dynamics are greatly influenced by agricultural management practices including crop rotation (Wright and Anderson, 2000) and tillage (Wright et al., 1999). Glomalin, a recalcitrant protein-like substance that has been linked to AMF, binds soil particles and contributes to aggregate stability (Wright and Upadhyaya, 1998). Glomalin is thought to confer water-stability by sealing macroaggregate pores and slowing the penetration of water into the aggregate (Rillig, 2004). Rillig and Steinberg (2002) simulated aggregated and non-aggregated soils using glass beads and showed that the AM fungi *Glomus intraradices* underwent little growth, but produced large quantities of glomalin in the non-aggregated material. In the simulated aggregated soil, hyphal growth dominated and little glomalin was produced. From this they inferred that AMF can manipulate their growth to facilitate the optimization of soil environmental conditions.

AMF are particularly sensitive to tillage disturbance (Wortman et al., 2008) and glomalin content of soils has been shown to increase under NT (Wright et al., 1999; 2007). Significant strides need to be made in our understanding of the nature and especially the origin of glomalin. However, it seems clear that at least some of this highly recalcitrant binding material is mycorrhizal in origin and that regardless of its nature, glomalin serves as a binding agent to bolster aggregation.

2.4.3 Aggregate stability and size distribution as a function of tillage

Tillage disrupts aggregates and results in a higher rate of macroaggregate turnover in CT than in NT soils (Six et al., 1999). Abundance of stable macroaggregates is greater in NT vs. CT soils (Wright and Hons 2005; Malhi et al., 2006; Singh and Malhi, 2006; Zibilske and Bradford, 2007; Alvarez and Steinbach, 2009) and is greatly influenced by organic matter build up near the soil surface (Arshad et al., 1999). Aggregate size distribution is frequently impacted by tillage, with greater macroaggregation at the soil surface in NT soils (0 to 5 cm), but there are seldom differences at greater depth (Bossuyt et al., 2002; Wright and Hons, 2005). This is important because under NT management, the longevity of macroaggregates leads to the formation of more stable microaggregates within their interior. Six et al. (2000) proposed a conceptual model that relates the protection of fine intra-particulate organic matter by microaggregates formed within macroaggregates as the major mechanism of stable C accumulation under NT.

2.4.4 Aggregate microbial communities

According to the Aggregate Hierarchy Theory, microorganisms are fundamental to the formation and stabilization of aggregates which, in turn, provide habitat for soil biota. Fungal

activity is essential to the formation of macroaggregates (Gupta and Germida, 1988; Bossuyt et al., 2001) providing transient binding agents including mycelium and polysaccharides. As such, both saprophytic and arbuscular mycorrhizal fungi are often positively correlated with the mass of macroaggregates (Six et al., 2006). A positive feedback mechanism between physical protection of recalcitrant cell wall residues (from microbial decomposition) within aggregates and aggregate stabilization by these cell wall residues has been proposed (Chantigny et al., 1997; Guggenberger et al., 1999). Bacteria also play an important role in aggregate formation at a smaller scale through their contribution of binding agents especially important for the formation of microaggregates. In this way, the composition of the microbial community within aggregates directly influences aggregate stability and longevity.

Spatial heterogeneity of resources among different aggregate size classes and within aggregates themselves give rise to an environment with enormous potential for colonization by a diverse community of microorganisms. Väisänen et al. (2005) showed that communities associated with larger aggregates had significantly faster respiration and differed in composition from microaggregate communities. Similarly, Ranjard et al. (2000) saw clearly distinctive bacterial communities associated with macro- vs. microaggregates. They hypothesized that in microaggregates genetic community structure was likely stable due to the stability of conditions in these microenvironments. Macroaggregate communities by contrast would be more likely to change with time and through space as a result of higher fluctuations in environmental conditions. Blackwood et al. (2006) found very high inter-aggregate variability among macroaggregate communities and did not detect differences in bacterial community structure between different macroaggregate sizes. They postulated that is the tertiary components of soil

structure (e.g., the proximity of aggregates to roots, plant residues, etc.) that is the more important determinant of macroaggregate community structure.

Changes in soil structure including increased aggregation under NT modify the microbial habitat in soil and likely alter the chemical environment at the microscale. In addition to altering aggregate size distribution, these changes in oxygen concentration and water content, coupled with increased contact between plant residues and particle surfaces within aggregates likely transform the microbial habitat. Lupwayi et al. (2004) measured greater bacterial functional diversity in NT than CT aggregates using C substrate utilization patterns (BIOLOG™). White and Rice (2009) used PLFA analysis to examine microbial community dynamics in residue amended NT and CT soils and concurrently examined aggregate size class distribution. They found increased microbial biomass in NT as well as greater amounts of residue-derived C and N in NT macroaggregates; however, microbial communities in different aggregate size classes were not included in their study. Peixoto et al. (2006) detected differences in the genetic bacterial community structure in bulk soils and concomitant differences in the distribution of water stable aggregates in NT and CT soils, but proposed that understanding microbial community structure within aggregate size classes is essential to understanding the effects of management on soil quality.

There is currently a gap in our understanding of aggregate dynamics due to a lack of research into microbial community dynamics in different aggregate sizes. Further work is needed to elucidate the mechanisms by which bacteria and fungi contribute to the storage of C within aggregate structures and how soil management affects aggregate microbial community structure.

2.4.5 Effect of NT on aggregate C and N

Storage of C and N in aggregates and among aggregate size classes changes as a function of tillage (Six et al., 2000; Wright and Hons, 2005). No-till management provides physical protection for labile soil C in macroaggregates (Six et al., 2000; Bossuyt et al., 2002; Mikha and Rice, 2004). Site-based differences in soil organic C accumulation under NT can be accounted for by aggregate dynamics, specifically aggregate turnover rates which determine the ultimate fate of newly introduced C within aggregates (Yoo and Wander, 2008). Simpson et al. (2004) found greater microbial-derived C stabilization in NT due to fungal-mediated improvements in soil structure and concurrent deposition of fungal-derived C in microaggregates within macroaggregates. Hu et al. (1995) similarly found that fungi were more important for the storage of organic C in NT than CT.

Total N storage in aggregates is greater under NT than CT (Mikha and Rice, 2004; Wright and Hons, 2005; Zibilske and Bradford, 2007). In addition to protection of organic matter, microbial cell wall residues contain significant N (Paul et al., 1997) and stabilization of these materials may contribute to increased N content in NT aggregates. Decreases in the C:N ratio of aggregates under NT have been observed (e.g., Zibilske and Bradford, 2007) and may be partially explained by the accumulation of N-rich microbial cell wall residues.

Changes in microbial community structure and function within aggregates, along with altered aggregate size distribution resulting from reduced tillage affect cycling of C and N although the exact mechanisms involved are not well understood. Broadly, inorganic N production increases as microbial activity and substrate concentration increase; however, C:N ratio has a negative effect on mineralization implying that soil organic matter composition also regulates N mineralization (Booth et al., 2005). It is likely that many of the tillage-induced

changes in nutrient cycling occur at the microscale and are enacted on aggregate surfaces or in aggregate interiors. Given what is known about the nature of C transformations within aggregates of differing longevity (Monreal et al., 1997; Paustian et al., 2000; Six et al., 2000; Yoo and Wander, 2009), it can be expected that the sequestration of C and N in aggregates in NT soils may profoundly affect N cycling. Greenhouse gas mitigation potential of NT must be approach from a net accounting perspective which accounts for all emissions and sinks. The net global warming potential of NT which reflects the balance between C sequestration and N_2O emissions must be carefully examined (Six et al. 2004b). The recent focus on NT for C sequestration has in some cases overshadowed the potential for other benefits of NT which extend beyond greenhouse gas mitigation. In this way, NT represents a "win-win" practice that promotes soil quality and sustainability in addition to having the potential for reducing the net environmental impact of agroecosystems. Enhancement of our understanding of the links between tillage, aggregation and C and N cycling is crucial in maintaining the sustainability of prairie cropping systems which are becoming increasingly based on principles of RT and NT.

2.5 Nitrogen dynamics in no-till systems

2.5.1 Soil nitrogen and availability for crop growth

Nitrogen dynamics in soil are complex and highly variable and despite decades of intense study, remain rather poorly understood. Interactions between inherent soil properties, climate and management influence N cycling in agroecosystems. Attempts to understand the effects of tillage on N cycling must be underpinned by a basic understanding of N cycling principles in a given system.

A decrease in N availability is often observed following conversion from CT to NT. This has been attributed to lower levels of mineralization of organic N protected by aggregates (Beare et al., 1994) and greater N immobilization under NT (Lupwayi et al., 2006a). In medium and fine textured soils, McConkey et al. (2002) observed decreased availability of soil N under NT. In their study, this was attributed to a slower rate of net N mineralization from organic matter. Once established, NT generally can provide a more favourable environment for microbial growth and in some cases, results in increased microbial biomass N and greater microbial activity increasing N turnover (Soon and Arshad, 2004). Resulting effects on crop yield are variable however. For example, in an 8-yr study of the effects of N fertilization, residue and tillage management in Saskatchewan, tillage had inconsistent effects on crop yields varying from year to year among different crop types (Malhi et al., 2006; Malhi and Lemke, 2007). Similarly, Soon and Arshad (2004) measured greater crop yield under NT in one of three years, whereas no significant effect of yield was detected in the remaining two years of their study. Effects of tillage on crop N uptake also showed similar variability (Soon and Arshad, 2004; Mahli et al., 2006; Malhi and Lemke, 2007).

Increased levels of NO_3^- (Grant and Bailey, 1994; Soon and Arshad, 2004; Lupwayi et al., 2006b) and total N (Grant and Lafond, 1994; Campbell et al., 1996b) at the soil surface in NT have been reported, but do not necessarily affect crop uptake (Lupwayi et al., 2006b). It has been speculated that build up of organic N that is subsequently mineralized from crop residues may be responsible for this vertical stratification of N at the soil surface. In addition to implications for crop yield, understanding N cycling in NT soils is important for minimizing losses to adjacent environments. Net greenhouse gas accounting of both soil C sinks as well as

potential emissions of CH_4 and N_2O is important for determining the impact of this prominent agricultural practice on our atmosphere.

2.3.2 Gaseous nitrogen losses

Sources of N_2O from soil include the processes of nitrification and denitrification. The proportion of N lost as N_2O during these processes is highly governed by microbial activity as a function of environmental factors such as soil moisture and temperature (Bouwman et al., 1993). Because these soil properties can be altered by tillage practices, emissions from NT and CT systems can be expected to differ. However, strong interactions among soil and environmental parameters make prediction of N_2O emissions as a function of management difficult. Risk of denitrification losses is often considered to be higher in NT due to the combined effects of increased soil moisture and higher bulk density on soil aeration (Aulakh et al., 1984).

In cultivated soils, NH_4^+ is generally present in relatively low concentration and nitrification is usually considered to be the major fate of NH_4^+. Nitrification is an aerobic process and O_2 is required for all nitrifying organisms. In this way, the change in moisture content and structure of soil under NT management likely affects nitrifying organisms and nitrification rate. Ammonium is the preferred N source for most soil microorganisms (Paul and Clark, 1996) and competition for this resource can influence nitrification rates. Soil organic matter (SOM) content largely influences the immobilization of N and increases in SOM under NT potentially increase the competition for ammonium between heterotrophic organisms and autotrophic nitrifiers.

The effect of tillage practices on the net emission of N_2O, a powerful greenhouse gas, is not clear. Some research has demonstrated that N_2O emissions are higher under NT than CT (Linn and Doran, 1984; Ball et al., 1999; Baggs et al., 2003) whereas others have found

emissions to be higher in more highly disturbed soils (Mahli et al., 2006; Malhi and Lemke, 2007). In yet other instances, comparisons of N_2O emissions as a function of tillage were less conclusive (Lemke et al., 1999). In a summary of N_2O emissions from NT and CT systems in Canada, Helgason et al. (2005) observed that although the absolute differences in emissions were highly variable, about half of the comparisons (56%) showed greater emissions in CT nationwide. Where N_2O emissions were measured for two or more years, the effects of tillage were reversed more than 70% of the time. While no statistically significant determination could be drawn, a general trend was observed that emissions often were higher from NT than CT in eastern Canada whereas the opposite held true in western Canadian studies.

Work by Six et al. (2004) determined that in fact, net reductions in global warming potential only result after long-term NT management due to trade-offs between C storage and N_2O emissions which are only beneficial over long time periods. Greater understanding of the pathways and ultimate fate of both C and N in NT soils is crucial for the long-term minimization of detrimental environmental impacts of agricultural production systems as well as the promotion of sustainable soils management.

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3.0 FUNGAL AND BACTERIAL ABUNDANCE IN LONG-TERM NO-TILL AND CONVENTIONAL-TILL SOILS OF THE NORTHERN GREAT PLAINS[1]

3.1 Preface

The relative proportion of bacteria and fungi in natural and managed soils can be starkly different. Soil disturbance in agroecosystems is thought to hamper fungi by breaking apart hyphal networks and thereby affecting fungal growth and establishment. No-till management minimizes soil disturbance, more closely mimicking disturbance levels in natural systems in which fungi are generally more dominant compared to typical agricultural soils. Fungi are able to adapt to the greater spatial heterogeneity of resources in NT soils, using their hyphal networks to bridge areas of high and low resource availability. Agroecosystem soil food webs are complex and fungi and bacteria play different and often complementary roles in decomposition. The overall size of the soil microbial biomass can be related to soil quality and the ratio of fungi to bacteria can affect potential rates and pathways of nutrient cycling. It is important to understand the effect of long-term NT management on the size and composition of the microbial biomass in order to understand the potential impact of this widely adopted management practice on agroecosystem sustainability. The objective of this study was to determine if long-term tillage management affected the overall size of the soil microbial biomass and the relative abundance of fungi vs. bacteria.

[1] This work has been previously published in Helgason, B.L., F.L. Walley and J.J. Germida. 2009. Fungal and Bacterial Abundance in Long-Term No-till and Intensive-till Soils of the Northern Great Plains. Soil Sci. Soc. Am. J. 73:120-127. Minor modifications have been made for consistency in formatting.

3.2 Abstract

Abundance of fungi and bacteria in long-term NT and CT soils in the Northern Great Plains were measured using phospholipid fatty acid analysis (PLFA) to determine if a shift in the relative abundance of fungi and bacteria occurs as the result of conversion to NT. Four tillage trials in each of four soil zones were sampled in spring of 2005 and 2006 before the crop was seeded to evaluate the long-term effect of tillage on the microbial community. With the exception of one site-year, total, bacterial and fungal PLFA were greater in NT than CT soils at the soil surface (0- to 5-cm depth) ($p<0.05$). Increases ranged from 8-202% for total biomass, 26-58% for bacterial biomass and 0-120% for fungal biomass. At one site (Ellerslie) all biomass measurements were greater in CT than NT in 2005 and bacterial biomass was also greater under CT in 2006. The influence of tillage on microbial biomass was less pronounced with depth. Fungal dominance is commonly assumed under NT; however, our results demonstrate that although biomass of both fungi and bacteria increase in NT, the abundance of fungi vs. bacteria was not consistently greater under NT in the soils studied. Further research is needed to determine if fungi may be able to exert a more functionally dominant role in NT soils without an increase in relative abundance.

3.3 Introduction

No-till management alters soil physical and chemical properties, creating a significantly altered habitat for bacteria and fungi. Under NT, crop residues are not incorporated into the soil and nutrients often become stratified near the soil surface. Sub-optimal environmental conditions (e.g., moisture, temperature) and diminished contact with soil particles leave surface placed residues less susceptible to microbial breakdown (Lupwayi, et al., 2004). This significant spatial change in resource distribution affects both the quantity and quality of substrate available for the microbial community in the soil profile.

Significant impacts of NT on soil biota, including both soil fauna and microorganisms have been well documented (Kladivko, 2001; Zibilske et al., 2007). For example, abundance of earthworms is greater in NT than CT (Wardle et al., 1995). In many cases, bacteria and fungi are also more abundant under NT than CT (Wardle et al., 1995; Kladivko, 2001; Pankhurst et al., 2002; Minoshima et al., 2007). Increases in microbial biomass C (Chantigny et al., 1997; Pankhurst et al. 2002; Lupwayi et al., 2004) and N (Soon and Arshad, 2004) under NT are generally greatest near the soil surface.

Soil conditions under NT may result in more frequent conditions of stress (e.g., lack of moisture at the soil surface) which are better tolerated by fungi than bacteria, (Dighton, 2003). Fungi are more adapted to growth at lower temperatures than bacteria (Pietikainen et al., 2005) providing a competitive advantage in NT as increased retention of residue on the soil surface keeps the soil surface cooler than in CT systems where residue is buried. In NT systems, fungal hyphal networks are left intact by the elimination of mechanical mixing that occurs during tillage events. Fungi can translocate nutrients through hyphal networks, often bridging air gaps and providing a physical connection between areas of low and high nutrient availability (Holland and

Coleman, 1987; Klein and Paschke, 2004). For example, Frey et al. (2000) demonstrated the ability of fungi to translocate N from the mineral soil upward into residue on the soil surface during decomposition in NT soils.

Studies in the US Great Plains indicated that under reduced and NT cropping systems there was a shift in the microbial decomposer community towards one dominated by fungi, particularly at the soil surface (Frey et al., 1999; Guggenberger et al., 1999). However, in an evaluation of fungal and bacterial dynamics in NT and CT soils in a corn cropping system in eastern Canada, Spedding et al. (2004) found no shift in fungal vs. bacterial abundance between tillage treatments. The objective of our study was to compare the abundance of bacteria and fungi CT and NT systems at four sites in the Northern Great Plains using a sampling strategy designed to maximize our ability to detect the long-term effect of tillage disturbance.

3.4 Materials and Methods

Long-term tillage experiments in four soil zones on the Canadian prairies were studied to determine the effect of tillage disturbance on soil fungal and bacterial communities. All sites used were replicated (n=4) randomized complete block designs (RCBD) with a paired treatment comparison of NT and CT (Table 1). Both crop rotation and the level of disturbance in the CT treatment varied at each site according to normal regional practices. Specifically, CT treatments decreased in intensity of disturbance as follows: Breton = Ellerslie > Scott > Swift Current. All sites were continuously cropped and crop rotation at each of the sites is outlined in Table 1.

Field soil was collected in at each of the four sites in two consecutive years (2005 and 2006). Soil sampling was performed once per year, prior to seeding at each site in order to minimize the effect of plant growth on the microbial community. Because of variable crop

Table 3.1 Descriptions of field sites for paired treatment comparison of no-till (NT) vs. conventional till (CT) at each of four long-term tillage trial locations.

Site	Soil order	Year initiated	pH	Texture	Crop rotation
Swift Current, SK	Brown Chernozem	1981	6.1	Loam	†wheat-lentil-wheat-pea
Scott, SK	Dark Brown Chernozem	1979	4.6	Loam	flax-wheat-wheat-canola-wheat-wheat
Ellerslie, AB	Black Chernozem	1980	5.2	Clay loam	triticale-pea-wheat-canola
Breton, AB	Gray Luvisol	1980	5.6	Loam	triticale-pea-wheat-canola

†Crop phase that is underlined indicates the crop grown in 2005.

rotations and intensity of tillage disturbance in the CT treatments, an inter-site comparison of the four locations was not valid. Therefore, only paired treatment comparisons of NT and CT at each site were performed.

3.4.1 Soil sampling methodology

Soil samples were collected using a JMC Backsaver probe (Clements Assoc. Inc, Newton, IA) equipped with a 3.175 cm diameter tip. Prior to sampling, any large crop residue segments were removed. Ten sub-samples (cores) per plot were taken (0- to 15-cm depth), and segmented into 5 cm increments in the field using a soil knife. The ten cores were then composited for each depth increment. Two additional core samples were taken for the determination of moisture content and soil bulk density (in 2005 only). Soils were stored on ice in a portable cooler during transport and processed (sieved to <2mm) within 48 h.

In 2005, field pea grown at Breton was destroyed by hail and no crop was harvested. A severe infestation of dandelion (*Taraxacum officinale*) followed in the NT plots, affecting our ability to measure tillage effects on the microbial communities at this site in the spring of 2006. As a result, PLFA data from Breton in 2006 was not included in our analysis.

3.4.2 Characterization of soil properties

Gravimetric and volumetric moisture content as well as bulk density were determined by measuring the moisture loss from soil core segments of a known volume after drying at 105 ºC for 48 h. In the first sampling year, soil particle size analysis was performed using the hydrometer method (Day, 1965) and soil pH was measured in a 2:1 soil:water slurry. Total C and N was determined by dry combustion using a LECO CNS-2000 analyzer (LECO Instruments,

Ltd., St. Joseph, MI) and available N (NO_3^- + NH_4^+) in a 1:10 soil:2 *M* KCl extract (colorimetric analysis).

3.4.3 Phospholipid fatty acid analysis

Phospholipid fatty acid analysis (PLFA) was used to assess relative biomass of fungi and bacteria. PLFA was performed using the modified method White et al. (1979), based on the original method of Bligh and Dyer (1959). Briefly, fatty acids were extracted from 4.0 g of lyophilized, ground soil. Fatty acids were separated on a solid phase extraction column (0.50 g Si; Varian Inc. Mississauga, ON), phospholipids were methylated and resulting fatty acid methyl esters were analyzed using a Hewlett Packard 5890 Series II gas chromatograph with a 25m Ultra 2 column (J&W Scientific). Peaks were identified using fatty acid standards and MIDI identification software (MIDI Inc., Newark, DE) and quantified based on the addition of internal standard methyl nonadecanoate (19:0). Total biomass was calculated as the sum of all identified PLFA peaks (Zelles et al. 1992). Fungal and bacterial biomass in NT and CT soils were assessed using the fungal biomarker 18:2ω6,9 and the sum of 13 bacterial biomarkers (i14:0, i15:0, a15:0, i16:0, 16:1ω7c, 10Me16:0, i17:0, a17:0, cy17:0, 10Me17:0, 18:1ω7, 10Me18:0, cy19:0) (Bååth and Anderson, 2003).

3.4.4 Data Analysis

Paired treatment comparisons of soil properties and microbial lipid biomarkers between NT and CT soils were made within each site. The long-term trials examined in this study were not replicated among the four locations making inter-site comparisons invalid. Analysis of variance (ANOVA) was performed using SPSS version 13.0 for Windows (SPSS Inc., 2004).

3.5 Results

This study assessed fungal and bacterial biomass in long-term NT vs. CT soils. Information presented is of particular value because consistent sampling was conducted in four different locations to maximize the capture of the effect of the tillage treatments while minimizing short-term plant or disturbance effects. All sites were sampled prior to seeding and spring tillage events (in the CT plots). Microbial abundance was measured using PLFA biomarkers which assess the relative abundance of viable biomass between tillage treatments (without bias from dead or partially decomposed microbial cells) (Pinkart et al. 2002; White et al. 1979). The use of PLFA is more sensitive than fatty acid methyl ester (FAME) analysis to changes in microbial community structure resulting from tillage–induced changes in soil environmental conditions (Petersen et al. 2002).

3.5.1 General soil properties

Soil characteristics differed at each of the four long-term sites (Table 3.1). Soil organic C and N (0- to 15-cm) were significantly greater in NT than CT at Scott and Breton, but were not different at Swift Current and Ellerslie (Table 3.2). When examined at each depth increment, NT soils had higher TC (total carbon) and TN (total nitrogen) in the 0- to 5-cm depth at Scott and Breton but not at Swift Current and Ellerslie (data not shown). At Breton, TC and TN were also greater under NT in the 10- to 15-cm depth. Where soil C increases under NT were measured, TN did not increase proportionally resulting in a wider C:N ratio in NT than CT at Scott and Breton. Conversely, the C:N ratio was significantly lower under NT than CT at Swift Current and Ellerslie where no significant change in TC was measured.

Table 3.2 General soil characteristics of four long-term tillage trials comparing no-till (NT) and conventional-till (CT) soils averaged across depths (0- to15-cm depth).

Site	Bulk Density	Total C	Total N	C:N
	($g\ cm^{-3}$)	($g\ kg^{-1}$)	($g\ kg^{-1}$)	
Swift Current				
NT	1.19	15.6	1.8	8.7*
CT	1.24	16.3	1.8	9.0
Scott				
NT	1.14	32.0*	3.1*	10.2*
CT	1.10	27.0	2.7	9.8
Ellerslie				
NT	1.05*	57.2	5.2	10.8*
CT	1.02	57.2	5.6	10.5
Breton				
NT	1.65	16.2*	1.5*	10.6*
CT	1.63	13.6	1.3	10.1

*Denotes a significant difference ($p<0.05$) between tillage treatments within a site.

Inorganic N (NO_3^- + NH_4^+) was also different among the four sites and varied between years. Similarly, inorganic N and tillage interacted with depth (Fig. 3.1; Table 3.3). At the 0- to 5-cm depth inorganic N was greater in NT than CT, but differences between tillage treatments were more variable and generally of less magnitude at other depths. Inorganic N levels were greatly elevated at Swift Current in 2005 and Ellerslie in 2006, especially in the 0- to 5-cm depth of the NT soil (Fig. 3.1).

3.5.2 Microbial biomass indicators

Total PLFA was greater in NT (ca. 60-90 nmol g^{-1} soil) than CT (ca. 40-60 nmol g^{-1} soil) in the 0- to 5-cm increment at all sites in both 2005 and 2006, with the exception of Ellerslie in 2005 (73 vs. 84 nmol g^{-1} soil) (Fig. 3.2). Total PLFA and tillage interacted with depth (Fig. 3.2; Table 3.3) but was consistently greatest at the soil surface.

The effect of tillage on bacterial PLFA was more complex, changing between years at individual sites and interacting with depth (Fig. 3.3). At Swift Current and Scott, bacterial lipids were greater in NT than CT at the soil surface (0- to 5-cm). At Ellerslie, the opposite was true. In 2005, bacterial PLFA at Ellerslie was not different between tillage treatments at the soil surface, however in 2006 the CT (19 nmol g^{-1} soil) treatment had greater bacterial PLFA than NT (11 nmol g^{-1} soil). Bacterial PLFA also decreased with depth at all sites.

At Swift Current and Scott, the fungal biomarker was greater in NT (ca. 2.4 nmol g^{-1} soil) than CT (ca.1.5 nmol g^{-1} soil) at the surface (0- to 5-cm) and decreased with depth (Fig. 3.4). This was also true of the fungal biomarker at Breton in 2005 (1.8 vs. 1.4 nmol g^{-1} soil). At Ellerslie, the fungal biomarker was higher in the CT than NT treatments in 2005 but in 2006 was more than 10-fold greater in the NT (25 nmol g^{-1} soil) than CT (2 nmol g^{-1} soil) soil (Fig. 3.4).

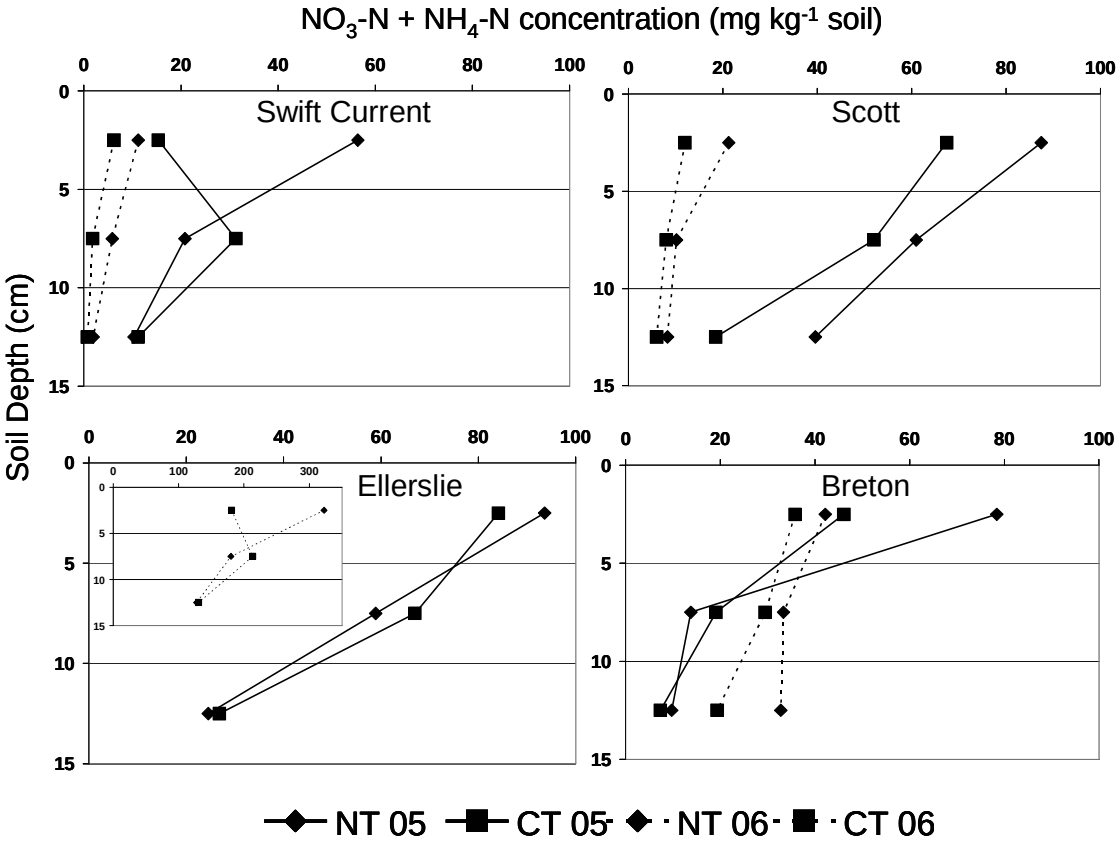

Figure 3.1 Inorganic N (NO_3^- + NH_4^+) (n=4) at three depth increments in long-term no-till (NT) and conventional-till (CT) soils measured in spring 2005 and 2006. Inset represents Ellerslie 2006 data.

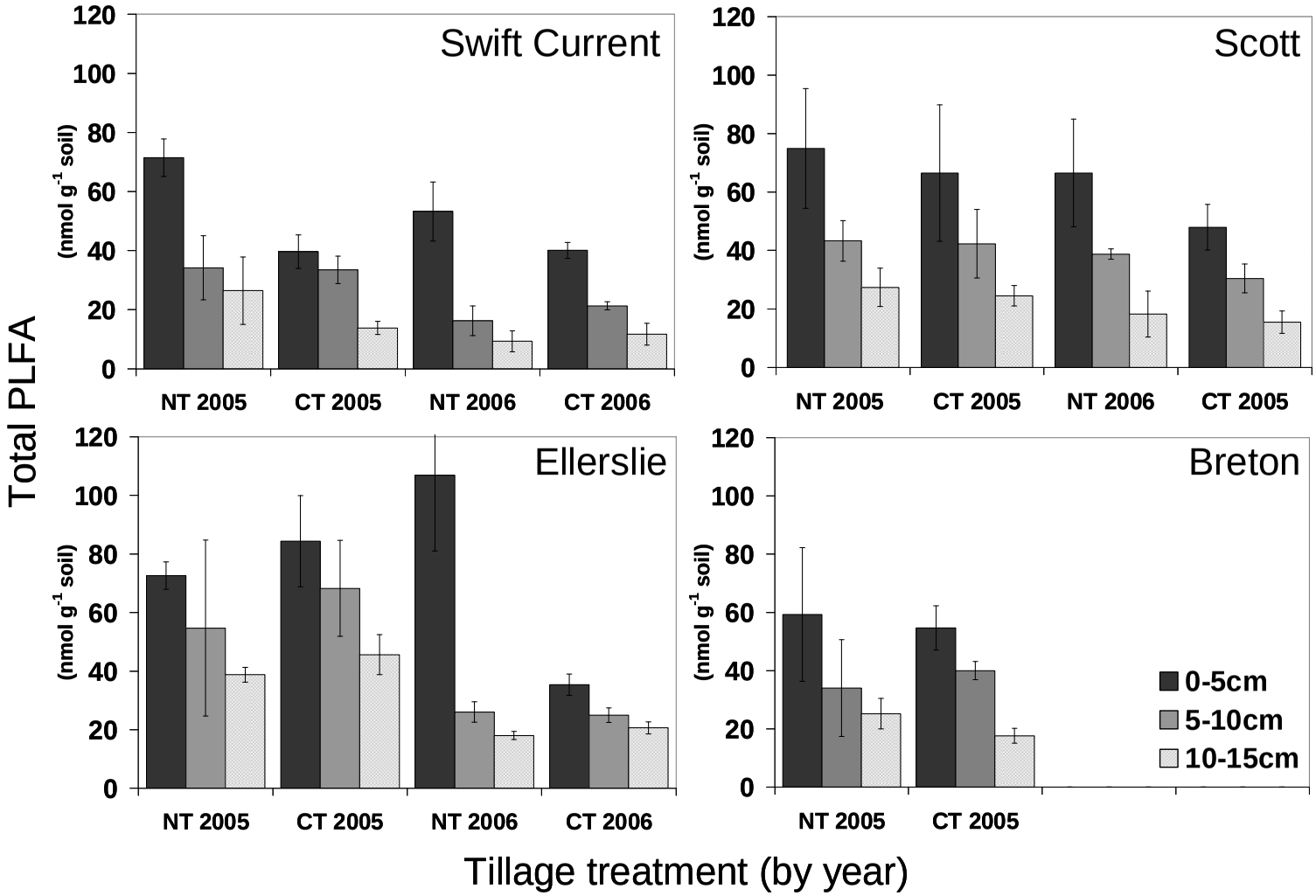

Figure 3.2 Total biomass (n=4) measured at three depth increments in no-till (NT) and conventional-till (CT) soils in 2005 and 2006 at four long-term tillage sites. Error bars represent standard deviation.

Table 3.3 Analysis of variance (ANOVA) of PLFA biomarker data for bacteria, fungi and fungal:bacterial ratio (F:B) at each of four long-term tillage sites.

	Swift Current			Scott			Ellerslie			Breton		
	Bacteria	Fungal	F:B	Bacteria	Fungal	F:B	Bacteria	Fungal	F:B	Bacteria	Fungal	F:B
Year	***	*	ns	**	ns	**	***	**	***	***	**	***
Tillage	***	***	**	ns	ns	ns	***	**	***	***	ns	ns
Depth	***	***	***	***	***	***	**	***	**	***	***	***
Year*tillage	*	**	*	ns	ns	ns	ns	**	***	***	**	ns
Year*depth	ns	ns	ns	ns	ns	ns	ns	**	**	ns	ns	ns
Tillage*depth	***	***	ns	ns	ns	ns	ns	**	**	ns	ns	**
Year*tillage*depth	ns	ns	ns	ns	ns	ns	ns	**	**	ns	ns	ns

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

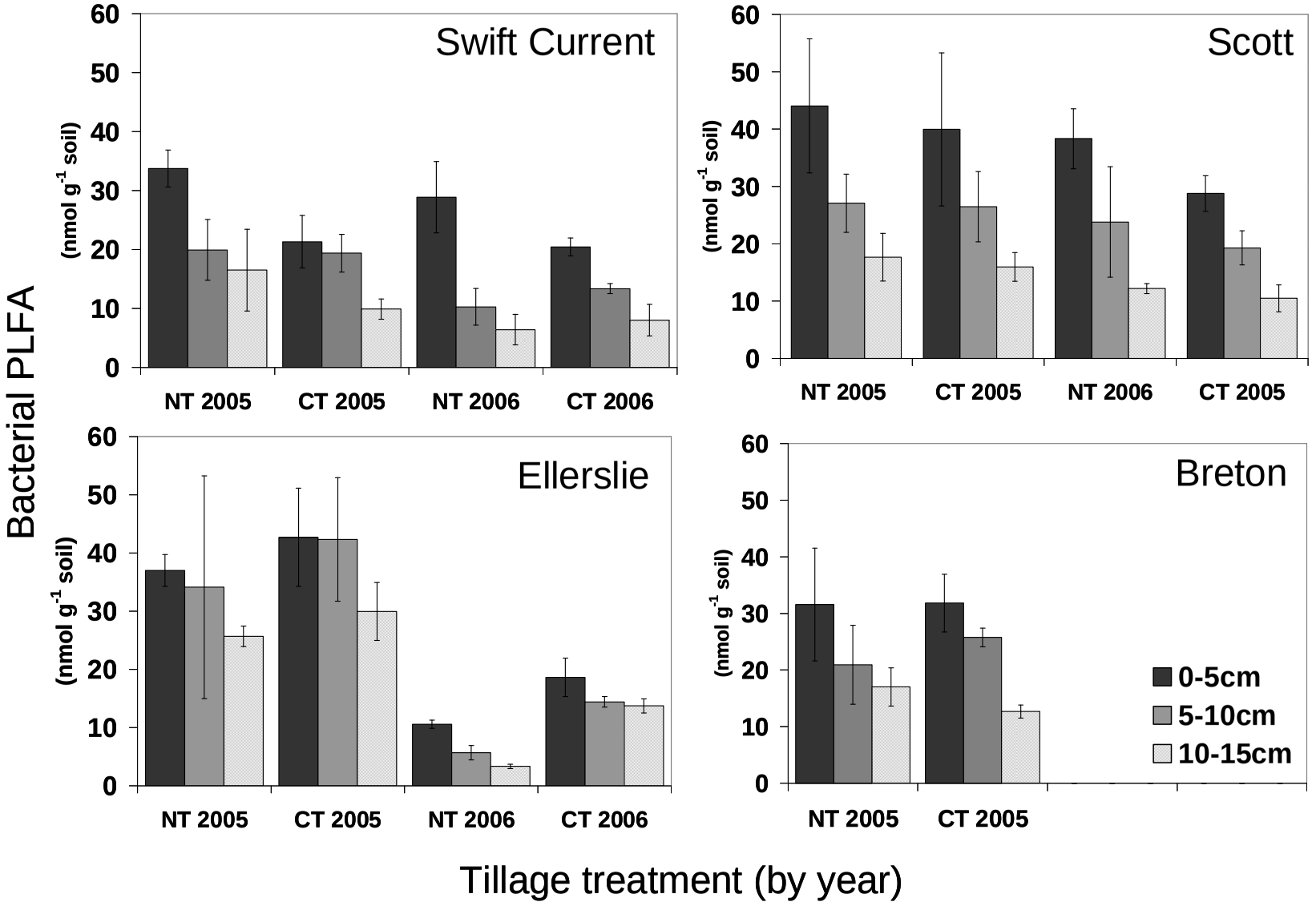

Figure 3.3 Bacterial biomass (n=4) measured at three depth increments in no-till (NT) and conventional-till (CT) soils in 2005 and 2006 at each of four long-term tillage sites. Error bars represent standard deviation.

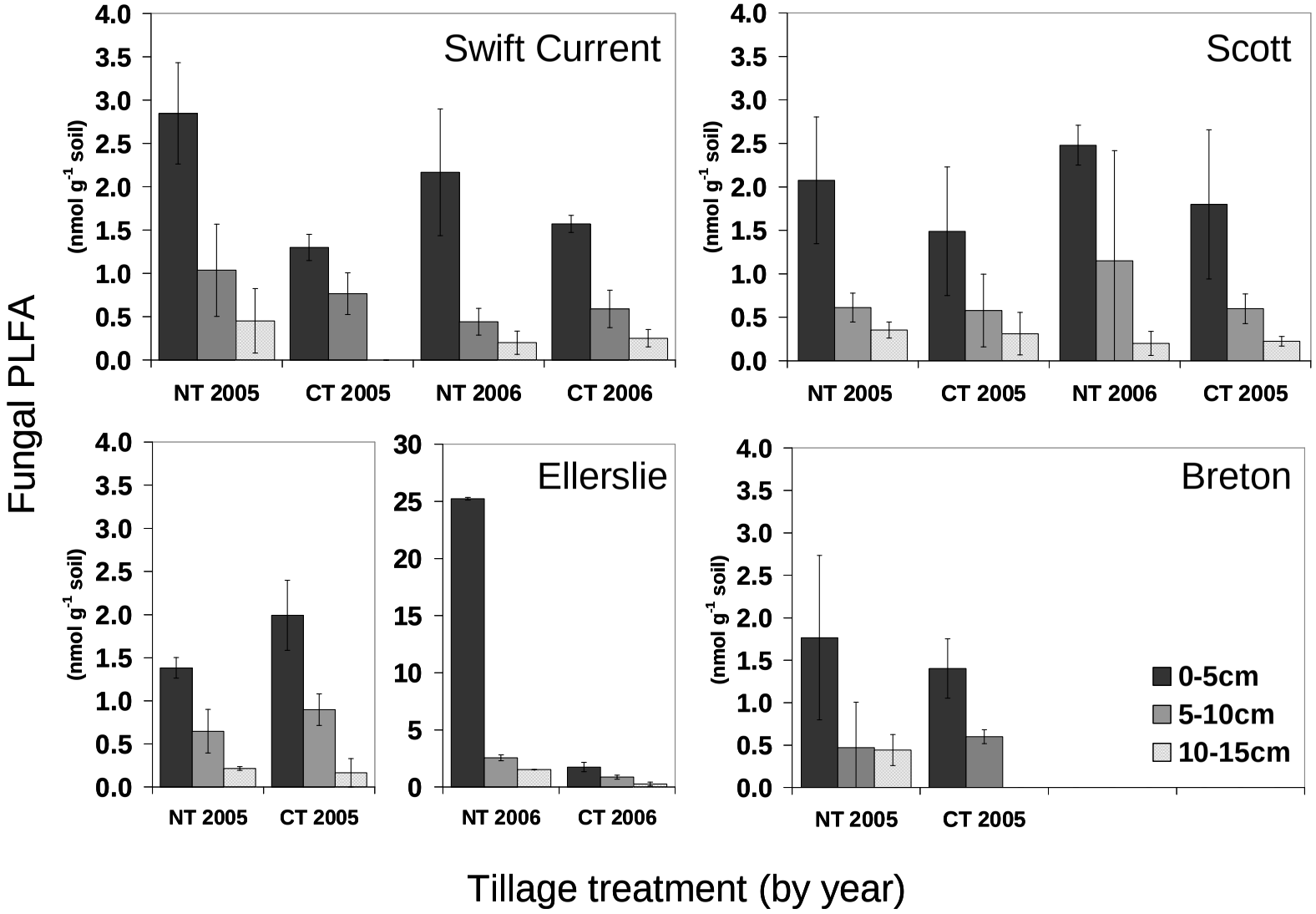

Figure 3.4 Fungal biomass (n=4) measured at three depth increments in no-till (NT) and conventional-till (CT) soils in 2005 and 2006 at each of four long-term tillage sites. Error bars represent standard deviation.

The effect of tillage on the fungal:bacterial ratio of PLFA biomarkers, indicative of the relative change in fungal vs. bacterial biomass (F:B), varied among years and locations (Fig. 3.5). At Swift Current there was an increase in F:B at the soil surface under NT in 2005 but not in 2006. At Scott there was no significant effect of tillage on F:B in either year. At Ellerslie in 2005 the F:B ratio was greater in CT than NT at the soil surface (0- to 5-cm) however this was reversed in 2006 when the F:B was much greater in the NT than CT soil. The drastic change in 2006 was due to both increased fungal PLFA and decreased bacterial PLFA in NT, relative to CT. At Breton, the F:B ratio was greater at 0- to 5-cm depth in NT than CT in 2005.

3.6 Discussion

Changes in soil characteristics following a conversion from CT to NT are generally associated with improved soil quality and enhanced sustainability of soil for crop production. Long-term continuous cropping in both CT and NT treatments and high inherent levels of organic C and N may have contributed to a lack of measured difference in these properties between tillage treatments at some sites.

Our observation of increased total microbial biomass at the soil surface in NT has been measured by other researchers in tillage systems from various geographical locations and agroecosystem types (Bailey et al., 2002; Drijber et al., 2000; Feng et al., 2003; Frey et al., 1999; Minoshima et al., 2007; Spedding et al., 2004). Lesser or negligible differences in microbial biomass between tillage treatments at greater depths were also in agreement with previous research.

Bacteria and fungi comprise more than 90% of the total soil microbial biomass (Beare, 1997). Our study demonstrated a consistent increase in PLFA biomarker for bacterial and fungal

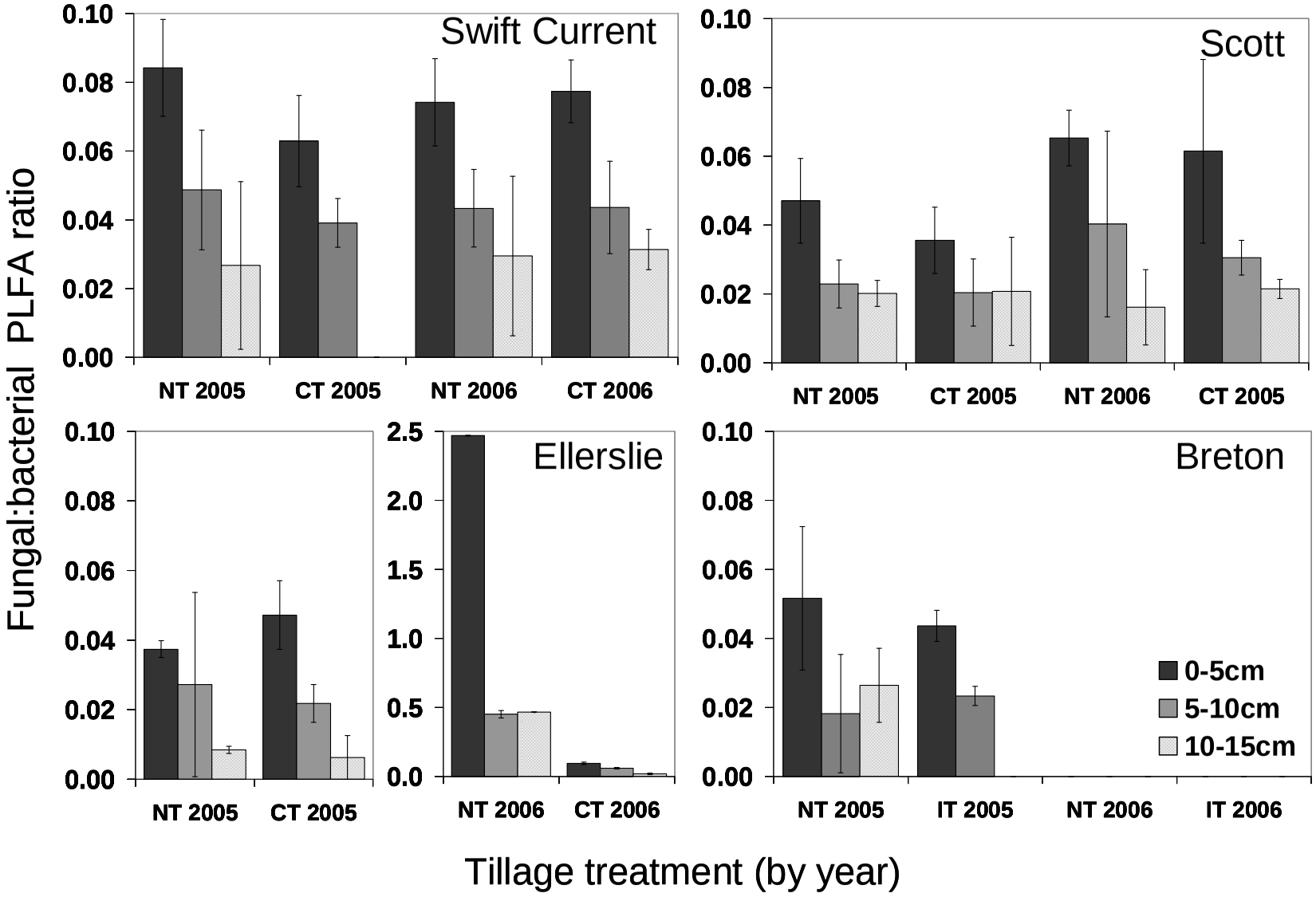

Figure 3.5 Ratio of fungal to bacterial biomass (n=4) measured at three depth increments in no-till (NT) and conventional-till (CT) soils in 2005 and 2006 at each of four long-term tillage sites. Error bars represent standard deviation.

abundance in NT vs. CT soils (with the exception of one site-year). These results are in agreement with other studies where biomass of both fungi and bacteria were observed to increase under NT (Drijber et al., 2000; Feng et al., 2003; Minoshima et al., 2007; Runion et al., 2004; Spedding et al., 2004). Nicolardot et al. (2007) demonstrated that resource quality affects the composition of the bacterial and fungal communities. In NT soils, the accumulation of residues on the soil surface results in nutrient stratification (Lupwayi, et al., 2006) providing a nutrient rich environment near the soil surface that can support increased microbial biomass. In addition, decreased physical disturbance in NT slows the rate of organic matter decomposition (Beare et al., 1994) and contributes to the longevity of microorganisms.

We did not observe a consistent shift toward fungal dominance as indicated by the ratio of fungal to bacterial biomarkers. This finding is in agreement with other comparisons of NT and CT management (Pankhurst et al., 2002; Spedding et al., 2004). Minoshima et al. (2007) observed an increase in both bacterial and fungal biomass and referred to a higher proportion of fungi in the microbial biomass under NT, but do not present the F:B ratio of their PLFA biomarkers. It was not apparent from our interpretation of their data that a change in the F:B ratio occurred. In contrast, Frey et al. (1999) found an increase in the abundance of fungi vs. bacteria under NT using staining and microscopy however fungal hyphal length assessments in their study may have been biased because the stain used (calcifluor M2R white) does not discriminate between viable and non-viable hyphae. If the NT environment provides conditions that are less conducive to breakdown of dead fungal hyphae, the use of a non-viable stain would result in an overestimation of fungal hyphal length in NT. Fungal cell wall constituents are also more resistant to breakdown than those of bacteria and may persist longer in soil following cell death.

However, PLFAs represent viable cells because they are rapidly degraded by phosphatases upon cell death (White et al., 1979).

No-till conditions provide an environment in which fungi can proliferate. Specifically, reduced physical disturbance and increased spatial heterogeneity of resources. While fungi are able to access spatially separated resources (Frey et al., 2000), Nicolardot et al. (2007) measured an effect of residue on fungal community structure on the surface of residue particles, but not in adjacent soil, indicating a minimal spatial effect of residue placement. Our results demonstrate that bacterial populations also thrive under NT and that the result was a proportional increase in the abundance of both communities. In the soils studied here, conditions created as a result of NT did not select for fungi at the expense of bacteria. It appears that both communities responded favourably and in equal proportion to the change in conditions.

Measures of microbial biomass do not necessarily indicate the activity or function of different groups within the soil microbial community. Much emphasis has recently been placed on the contributions of fungi and bacteria to aggregation and SOM accumulation in soils. The importance of fungi on soil structure and development has long been acknowledged. For example, Simpson et al. (2004) found greater microbial amino sugar C in NT than CT soils, which was attributed to the fungal-mediated improvement in soil structure and deposition of fungal C within aggregates. Six et al. (2006) discuss contributions of bacteria and fungi to C storage in soil. While the importance of fungi for the development and maintenance of soil organic matter was stressed, a lack of understanding was acknowledged regarding the mechanisms which facilitate this quantitative and qualitative improvement of SOM. They proposed that differences in the biochemical and chemical stabilization of organic matter derived

from fungi and bacteria seem to differ. In addition, they argue that differences in aggregate turnover as a result of fungal influences are important for C-retention.

Our observations of soil microbial biomass dynamics in NT soils do not disagree with these principles of SOM dynamics, but it is important to stress that we did not observe a *relative* increase in the abundance of fungi vs. bacteria in NT soils. Theoretically, if fungal biomass differentially affects the quality or quantity of soil organic matter (e.g., by protecting SOM within macroaggregates or through greater recalcitrance of fungal decomposition by-products) then each incremental increase in the absolute amount of fungal biomass will result in a proportionally greater effect on SOM accumulation than a corresponding increase in bacterial biomass. For example, Beare et al. (1992) demonstrated that litter decomposition was slowed by fungal inhibition to a greater extent in NT surface litter compared to CT buried litter. They also demonstrated the prevalent role of fungi for cycling N in NT soils. Acosta-Martínez et al. (2007) similarly showed that FAME fungal biomarkers were correlated with soil enzyme activity. Therefore, the end result may remain that fungi are more important to SOM dynamics in NT than CT systems and are key drivers of C accumulation under NT.

3.7 Conclusions

NT management results in increased bacterial and fungal biomass at the soil surface. No consistent shift toward greater relative abundance of fungi vs. bacteria was observed in the long-term NT soils studied. We suggest that the common generalization that NT systems are fungally-dominated is tentative.

Current interest in the relationship between soil fungi and organic matter dynamics shows great promise for the determination of specific causal relationships through which fungi may

qualitatively and quantitatively influence SOM. No-till systems provide an ideal environment for studying fungal contributions to soil development in agroecosystems because the selective pressure traditionally imposed against fungi by tillage disturbance is removed. Under these circumstances, fungi may have increased importance for improvements in soil organic matter, but further study is required to better understand the specific functional relationships between the soil biota and their environment in response to NT.

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4.0 LONG-TERM NO-TILL MANAGEMENT IS NOT AN OVERRIDING DETERMINANT OF MICROBIAL COMMUNITY DYNAMICS IN PRAIRIE AGROECOSYSTEMS

4.1 Preface

Previous studies have found that fungi dominate over bacteria in NT compared to CT soils. Furthermore, the argument is often made that because of the dominance of fungi in NT soils, microbial community structure is likely to change because of altered patterns of crop residue decomposition. The previous chapter examined the effect of long-term NT vs. CT management on the overall size of the microbial biomass as well as the relative biomass of fungi to bacteria. NT resulted in an increase in the total biomass as well as in the biomass of both bacteria and fungi, compared to CT but there was no consistent shift in the relative abundance of these two broad groups. However, surface deposition of crop residues results in a vertical stratification of nutrients and organic matter, potentially affecting microbial community composition. Lack of physical mixing of the soil in NT may further exacerbate this vertical stratification. Thus, despite the lack of a consistent shift in F:B ratios, potential exists for the formation of different microbial communities in long-term NT and CT soils. Therefore, the objective of this study was to determine if long-term NT vs. CT management affected microbial community composition and phylogenetics of bacterial and fungal populations.

4.2 Abstract

Microbial diversity can be used to assess the relative impact of management on the long-term sustainability of cropping systems. No-till management greatly reduces soil physical disturbance and can result in a stratification of nutrients and organic matter in the soil profile due to the retention of crop residues on the soil surface potentially affecting the dynamics of microbial interactions in the soil. The objective of this study was to assess the impact of long-term NT vs. CT management on soil microbial community structure at four different sites on the Canadian prairies. Analysis of 16S and 18S rDNA revealed high inherent variability within bacterial and fungal community fingerprints among replicate field plots. Differences in bacterial and fungal phylogeny were related to depth in the soil profile but not to tillage management. Individual PLFA biomarkers were 7 to 86% higher in NT surface soils (0- to 5-cm depth), except at the Ellerslie site in 2005 where biomass was greater in CT. Responses at the 5- to 10-cm and 10- to 15-cm depths were more varied, often with greater biomass in CT than NT soils. Ordination analysis of PLFA profiles showed clear community separation with depth but not tillage, in most cases. Physiological stress biomarkers were correlated with nutrient concentrations and indicated that resource availability was the main factor determining community structure. It was concluded that tillage disturbance was not an overriding factor in determining microbial community composition in the long-term NT and CT soils studied.

4.3 Introduction

Microorganisms interact with physical and chemical factors of soil through a series of feedback mechanisms that affect crop growth. This intricate system of interactions between biotic and abiotic factors bestows the foundation of microbial diversity in soil. Soil biota are vital to agroecosystem functioning and sustainability through their contributions to biogeochemical cycling and the provision of maintained fertility. Understanding how cropping system management affects the structure of microbial communities can provide enhanced insight into nutrient cycling processes and sustained productivity of agricultural systems.

Phylogenetic and functional diversity are often used as indicators of soil quality and fertility in agricultural soils. While relationships between phylogenetic and functional diversity can be difficult to link directly, it has been shown that soil microbial communities with high diversity (evenness) have greater resilience to stress (Zhou et al., 2002). This provision of resistance to perturbation makes functional diversity of soil microbes fundamental to agroecosystem sustainability (Giller et al., 1997). Agricultural intensification and increased global demand for food are increasing our reliance on the soil resource for provision of nutrients for food production. Understanding how major shifts in land management affect soil microbial community structure can provide an important index for assessing the relative ability of soils to respond to future perturbation.

No-till (NT) is an agricultural management practice that reduces physical soil disturbance and therefore should affect habitat for soil organisms. No-till can alter soil moisture and temperature regimes (McConkey et al., 1996; Soon and Arshad, 2004; Neilsen et al., 2005) and affects spatial resource distribution (Grant and Bailey, 1994; Lupwayi et al., 2006), aggregation (Six et al., 2002; Alvarez and Steinbach, 2009), nutrient cycling (Cookson et al., 2008; Triplett

and Dick, 2008) and ultimately crop growth. Within the Canadian Prairies, elimination of soil disturbance through RT and NT has been widely adopted as a means to reduce erosion, enhance organic matter accumulation and conserve moisture. Most agricultural land is managed under some form of RT (70%) and over 50% of seeded acres are managed with NT (Stats Can, 2008).

Microbial biomass accumulation under NT, primarily at the soil surface, has been demonstrated in most instances (Frey et al., 1999; Feng et al., 2003; Spedding et al., 2004; Acosta-Martinez et al., 2007; Helgason et al., 2009; White and Rice, 2009). However, in some cases this effect is not observed (Drijber et al., 2000; Carpenter-Boggs et al., 2003), indicating that the underlying mechanisms that drive biomass accumulation in NT are linked to a broader suite of factors than simple physical disturbance. Measures of microbial communities in NT vs. CT soils indicate a greater functional diversity of the bacteria in NT (Lupwayi et al., 1998; Acosta-Martinez et al., 2007; Cookson et al., 2008). However, most of these assays are based on the culturable, heterotrophic bacterial component of the microbial community. Fewer studies have been conducted using culture-independent, and especially nucleic acid based examinations, of microbial community dynamics as a function of tillage.

Assessments of the short-term effects of tillage have shown immediate but short-lived effects of physical disturbance on microbial community structure (Calderon et al., 2001; Jackson et al., 2003; Wortman et al., 2008). Differences in microbial community dynamics, including increased bacterial and fungal biomass has been demonstrated as early as the first year following a conversion from CT to NT (Minoshima et al., 2007). However not all studies found an effect of tillage disturbance (Drijber et al., 2000; Petersen et al., 2002; Carpenter-Boggs et al., 2003; Spedding et al., 2004). Microbial community dynamics in NT soils may vary as a function of soil type, crop rotation and length of time that soils have been managed under NT.

Assessments of tillage-induced changes in porosity and organic matter are most consistent when measured a minimum of 15 y following conversion to NT after soils have had sufficient opportunity to become adapted or perhaps equilibrated under NT management (Kay and VandenBygaart, 2002). Since soil organic matter and microbial community dynamics are intricately linked, it is likely that they share common factors that control variability through time. Similar to soil C measurements, more representative assessments of tillage-induced effects on the soil microbial community are likely to be found in long-term experimental sites.

Comparisons of tillage intensity made during the growing season are often masked by the temporal effects of crop growth on the microbial community (Drijber et al., 2000; Feng et al., 2003; Spedding et al., 2004). Spatial and temporal heterogeneity of resources in soil result in soil microbial populations that are inherently tailored to coexist (Torsvik and Øvreås, 2002) and different subsets of the overall community are more active than others, depending on factors that vary temporally. Our approach was to best capture the tillage-induced effects on microbial communities by sampling in spring, immediately prior to seeding when the communities were least likely to be affected by plant growth or short-term disturbance effects.

The objective of this study was to determine if increased total, bacterial and fungal biomass in NT vs. CT soils (Ch. 3; Helgason et al., 2009) was accompanied by a shift in microbial community structure in long-term NT soils located at four sites in western Canada.

4.4 Materials and Methods

Long-term (>25 y) tillage experiments in four soil zones on the Canadian prairies were studied to determine the effect of tillage disturbance on soil fungal and bacterial communities (Figs. A.1 and A.2). All experiments at the four sites used replicated (n=4) RCBD with a paired

treatment comparison of NT and CT. Productivity and common cropping practices are highly variable among sites. Both crop rotation and the level of disturbance in the CT treatment varied at each site according to normal regional practices and all sites were continuously cropped (Table 3.1).

4.4.1 Soil sampling methodology

Field soil was collected for two consecutive years at each of the four long-term sites (2005 and 2006). Soil sampling was performed once per year, prior to seeding at each site. Soil samples were collected using a JMC Backsaver probe (Clements Assoc. Inc, Newton, IA), equipped with a 3.175 cm diameter tip. Prior to sampling, any large crop residue segments were removed. Ten sub-samples (cores) per plot were taken, and segmented into 5-cm increments in the field using a soil knife. Two additional core samples were taken for the determination of soil bulk density (in 2005 only) and moisture content. Soils were stored on ice in a portable cooler during transport and processed (sieved to <2 mm) within 48 h. Soil for PLFA analysis was freeze-dried and ground with a mortar and pestle to maximize lipid recovery (Allison and Miller, 2005). Total C and TN were determined by dry combustion using a LECO CNS-2000 analyzer (LECO Instruments, Ltd., St. Joseph, MI). Mineral N (NO_3^- + NH_4^+) was extracted in a 1:10 soil:2*M* KCl and determined by colorimetric analysis. Soil pH was measured in a 2:1 soil:water slurry

4.4.2 Phospholipid fatty acid analysis

Phospholipid fatty acid analysis was used to assess biomass relative abundance of microbial functional groups and was performed using the modified method of White et al.

(1979), based on the original method of Bligh and Dyer (1959). Briefly, fatty acids were extracted from 4.0 g of lyophilized, ground soil. Fatty acids were separated on a solid phase extraction column (0.50 g Si; Varian Inc. Mississauga, ON); neutral and phospholipids were methylated and resulting fatty acid methyl esters were analyzed using a Hewlett Packard 5890 Series II gas chromatograph with a 25m Ultra 2 column (J&W Scientific). Peaks were identified using fatty acid standards and MIDI identification software (MIDI Inc., Newark, DE) and quantified based on the addition of a known concentration of the internal standard methyl nonadecanoate (19:0). Biomarker abundance was calculated based on the peak area detected for each fatty acid, relative to that of a known quantity of the internal standard. Values are reported on the basis of soil dry mass. Biomarkers used to represent gram positive bacteria (Gr+) were i14:0, i15:0, a15:0, i16:0, i17:0, a17:0 (Hedrick et al., 2005). Biomarkers used for gram negative bacteria (Gr-) were 16:1ω7t, 16:1ω9c, 16:1ω7c, 18:1ω7c, 18:1ω9c, cy17:0, and cy19:0 (Macdonald et al., 2004). Abundance of arbuscular mycorrhizal fungi (AMF) was evaluated using the PLFA biomarker 16:1ω5c (Olsson, 1999). Physiological stress biomarkers denoted as Stress 1 and Stress 2 represent the ratios of cy17:0 to 16:1w7c and cy19:0 to 18:1w7c, respectively (Grogan and Cronan, 1997).

4.4.3 Analysis of fungal and bacterial species richness using DGGE

DNA was extracted from 0.75 g of field soil using the MoBio Ultra Soil DNA extraction kit (MoBio Laboratories Inc., Carlsbad, CA). Community structure and taxonomic diversity of the soil microbial community was examined by PCR amplification of the total DNA extracts and DGGE. A PCR using 18SrDNA primer pairs NS1 (5’- GTA GTC ATA TGC TTG TCT C -3’) and EF3 (TCC TCT AAA TGA CCA AGT TTG) (~1800 bp fragment) nested with FR1-gc

(CCC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GCC G _AIC CAT TCA ATC GGT AIT) and NS1 (~1685 bp fragment) was used to assess the fungal species richness (Oros-Sichler et al., 2006). PCR-amplification using 16S rRNA primers U341-gc (5'-GCG GGC GGG GCG GGG GCA CGG GGG GCG CGG CGG GCG GGG CGG GGG _CCT ACG GGAGGC AGC AG-3') and U758 (CTACCAGGGTATCTAATCC) (~417 bp fragment) were used to assess bacterial species richness (Rölleke et al., 1996; Phillips et al., 2006).

Fungal PCR reactions were carried out in a volume of 20 µL using 1 µL of 1:10 diluted DNA extract, 1.25 µ*M* each primer, 1.25 µg BSA (Amersham Biosciences) and 10 µL of Taq Master Mix (Qiagen). PCR for the EF3/NS1 amplification was performed with an initial denaturing step of 5 min at 94°C followed by 25 cycles of 30 s denaturing at 94°C, 45 s annealing at 47°C and 3 min extension at 72°C and a final annealing step of 10 min at 72°C. PCR for the FR1-gc/NS1 amplification was performed with an initial denaturing step of 5 min at 94°C followed by 25 cycles of 30 s denaturing at 94°C, 45 s annealing at 48°C and 3 min extension at 72°C and a final annealing step of 10 min at 72°C.

Eubacterial PCR reactions were performed in a reaction volume of 50µL consisting of 1 µL of 1:10 diluted DNA extract, 0.5 µ*M* each primer, 200 µ*M* each dNTP, 1.65 m*M* $MgCl_2$, 6.25 µg BSA (Amersham Biosciences) and 2.5 U Taq Polymerase in 5.0 µL 10X reaction buffer (Invitrogen). Touchdown PCR was used to minimize non-specific priming (65°C to 55°C) and amplification was performed for 10 cycles of 1 min denaturing at 94°C, 1 min annealing at 65 to 55°C and 1 min extension at 72°C. This was followed by 20 cycles using an annealing temperature of 55°C.

For both fungal and bacterial fragments, product amplification was confirmed by electrophoresis on ethidium bromide stained 1.0% agarose gels. Combined PCR reaction

products were concentrated by 0.1 V 3*M* sodium acetate and 2.5 V 70% ethanol at -20°C overnight and re-suspended in TE buffer. Final DNA concentration was determined by visual comparison with a Low Mass ladder (Invitrogen) on a 2.0% agarose gel stained with ethidium bromide.

Community structure was evaluated using DGGE analysis (Muyzer et al. 1993) of 18S rRNA and 16S rRNA gene fragments on a Bio-Rad DCode system (Bio-Rad, Mississauga, Ont.). For fungal community analysis, approximately 500 ng of DNA was loaded for each treatment onto an 8% acrylamide gel with a 25 to 45% urea-formamide denaturing gradient. Electrophoresis was performed for 18 h at 180V and 58°C. For bacterial community analysis, approximately 600 ng of DNA was loaded for each treatment onto an 8% acrylamide gel with a 40 to 60% denaturing gradient. Electrophoresis was performed for 16 h at 80V and 60°C. The resulting gels were stained with Sybr Green 1 (Sigma) and visualized using a GelDocMega gel imaging unit (Biosystematica, Ceredigion, UK). Random dominant bands were excised from the gel using a sterile scalpel, vortexed briefly with sterile glass beads and eluted in water for 45 min at 37°C. DNA was re-amplified using the primers U341 and U752 under conditions of 25 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 64°C and extension for 1 min at 72°C with a final extension of 3 min at 72°C. Amplified fragments were confirmed on 2% agarose gel, excised and purified with the GeneClean II Kit (QBiogene, Carlsbad, CA) following manufacturer's instructions. DNA sequences were submitted for comparison to GenBank databases to confirm fungal and bacterial origins using the BLAST algorithm (Altchul et al., 1997).

Gel analysis and band detection as well as cluster analysis was performed in Bionumerics v.5.1 (Applied Maths, Austin, TX). Selection of DGGE bands was done using a minimum

profiling of 5.0%, a position tolerance of 1.5% and with optimization of 2.0%. Cluster analysis was performed using the Pearson correlation coefficient based on densiometric curves and the Ward linkage method. Densiometric curves were then used to perform bandmatching, creating a binary presence-absence matrix (Boon et al., 2000; Peixoto et al., 2006). This binary matrix was subsequently subjected to non-metric multidimensional scaling (MDS) analysis (Smith et al., 2008) which was performed in PCOrd as outlined below.

4.4.4 Statistical analysis

Ordination of PLFA data (mol %) and DGGE banding pattern data by MDS using the Sørensen distance measure was carried out in the Autopilot Slow and Thorough analysis option (McCune and Grace, 2002) in PCOrd v.5.0 (MjM Software Gleneden Beach. OR). MDS is a nonparametric ordination method which robustly handles non-normally distributed ecological data (Clarke 1993; McCune and Grace, 2002). A random starting point was used for initial testing, but for the final ordination the starting configuration was supplied from a previous run in which the final stress had been minimized. Significance testing using Monte Carlo analysis was also performed. Multi-response permutation procedures (MRPP) was performed using the Sørensen distance measure to test for differences between *a priori* groups and was carried out in PCOrd v.5.0. For PLFA ordinations comparing overall effects of depth and tillage at all sites, 2006 data from Ellerslie and Breton were removed due to undue influence of the fungal biomarker at Ellerslie and the previously mentioned crop failure and subsequent weed infestation at Breton.

Analysis of variance was performed using SPSS Version 13.0 for Windows (SPSS Inc., 2004). Prior to analysis, PLFA data was transformed using the log(mol % + 1) transformation

and were presented as untransformed data. ANOVA was performed on paired treatment comparisons within each site but not among sites because experiments were not replicated across all sites. Significant differences are reported for those means that differed at the $P<0.05$ level, unless otherwise indicated.

4.5 Results

Microbial community structure in NT and CT soils was compared using molecular and biochemical approaches. Broadly, community fingerprinting of soil fungi and bacteria using 18S rDNA and 16S rDNA DGGE analysis showed differences at each site as a function of depth, but to a lesser extent as a function of tillage (Figs. 4.2; 4.7; 4.8). At individual sites, variability among replicate field plots was quite high within a given depth increment which resulted in differences that were frequently greater among replicate plots than between tillage treatments.

Overall biomass of AMF, Gr+ and Gr- bacterial was greater at the soil surface in NT soils. Proteobacteria and Actinomycetes biomass in NT and CT soils was similar to that of AMF and Gr+ and Gr- bacteria (data not shown). Ordination and MRPP analysis of the PLFA profiles of microbial communities at all sites showed that tillage did not have a significant effect on the overall community structure (Fig. 4.1). However, depth was a significant determinant of community composition across these spatially and agronomically distinct sites and was primarily differentiated along Axis 1 which accounted for 65% of the variability in PLFA profile data. Axis 2 explained a further 26% of variability in the PLFA profiles in these soils and was related to site differences, with Scott profiles forming a distinct cluster (Fig. B.7). Specific comparisons at each site and of tillage within each depth increment at each site similarly showed no significant effect of tillage and a strong effect of depth on microbial community composition

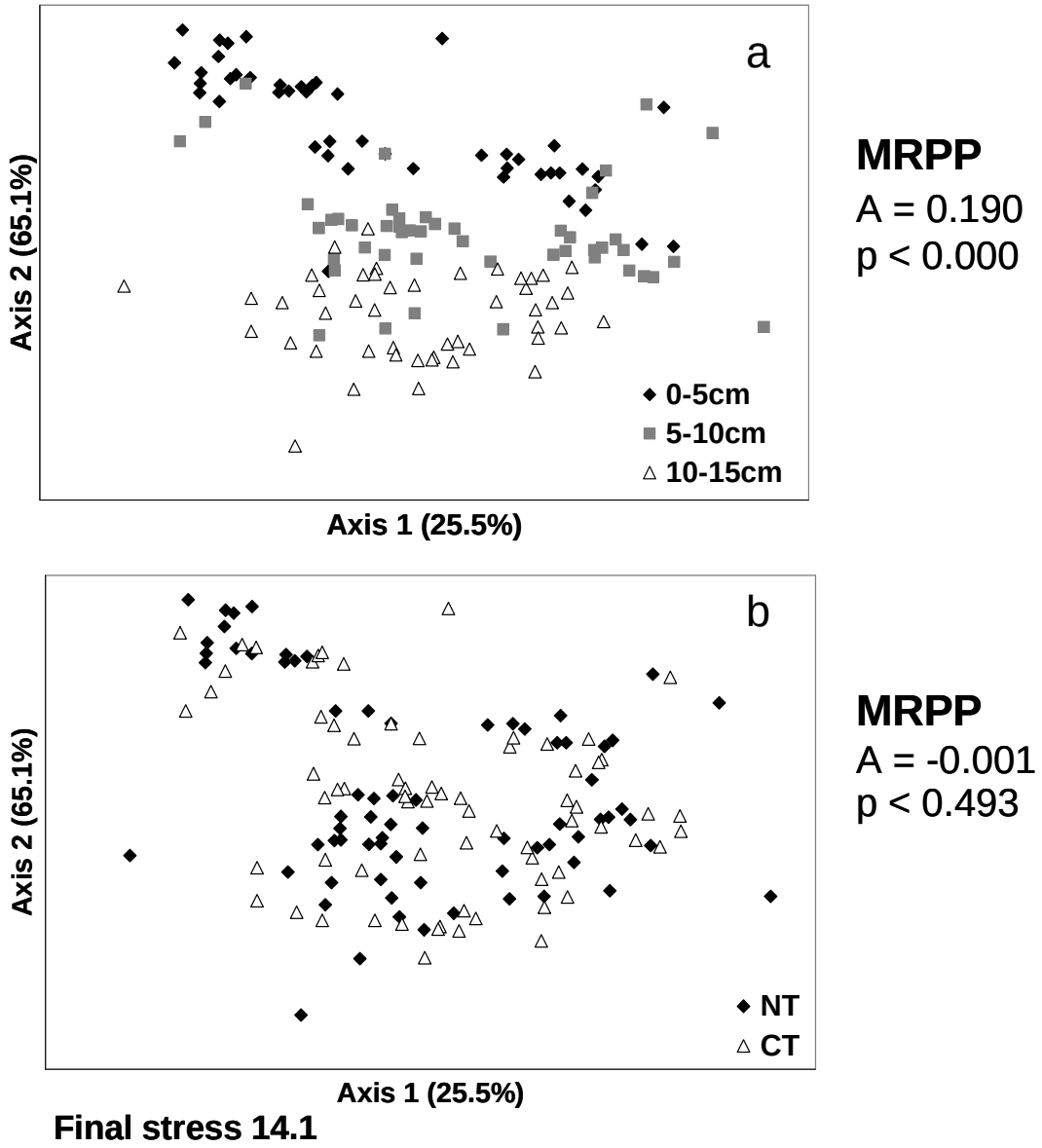

Figure 4.1 Non-metric multidimensional scaling (MDS) analysis (final stress = 14.1) and multiple response permutation procedure (MRPP) analysis of depth (a) and tillage (b) effects on PLFA profiles (mol % data) from 2005 and 2006 field soils at four long-term tillage research sites.

(data not shown). Soil TC, TN and C:N ratio are listed in the previous chapter in Table 3.2 and mineral N (NO_3^- and NH_4^+) was depicted in Fig.3.1. Correlation of PLFA data with soil physical or chemical characteristics data revealed a significant relationship between mineral N (NO_3^- + NH_4^+) and depth. Total C, TN and mineral N were positively correlated with microbial biomass and negatively correlated with the physiological Stress 2 biomarker (Table 4.1) at Swift Current, Scott and Breton indicating that stratification of resources is likely a dominant controller of community structure. The general lack of correlation between these parameters at Ellerslie which had very high TC and TN as well as mineral N contents was likely due to the fact that these resources were not limiting at this site.

4.5.1 Site specific observations

4.5.1.1 Swift Current

MDS analysis of 18S rDNA fingerprints at Swift Current resulted in a sub-optimal ordination with a high final stress value of 24.3 and little differentiation of fungal communities as a function of depth or tillage (Fig. 4.2A). Surface soils clustered somewhat along Axis 1, but overlapped with 5- to 10-cm depth soils. There was no separation as a function of tillage in this ordination analysis, which was further confirmed by cluster analysis of 18S rDNA fingerprints at the 0- to 5-cm depth (Fig. 4.2A) and at the 5- to 10-cm and 10- to 15-cm depths (Fig. B.1).

Ordination analysis of 16S DGGE fingerprints resulted in a 3-dimensional solution (final stress = 12.7), with the majority of variability accounted for in Axis 2 (66%). Axis 1 and 3 accounted for 12% and 10% of the variability respectively, and did not provide any obvious differentiation of tillage or depth. Similar to fungal DNA analysis, tillage did not have an overwhelming effect on bacterial community structure, but there was a clear effect of depth

Table 4.1 Pearson correlation coefficients between PLFA physiological stress biomarkers and soil mineral N (NO_3^- + NH_4^+), total C (TC), total N (TN) and AMF, gram positive and gram negative bacterial biomarkers.

	NH_4^+ + NO_3^-	TC	TN	AMF	Gram positive	Gram negative
Swift Current						
Stress 1	-.514***	-.646***	-.593***	-.711***	-.659***	-.672***
Stress 2	-.465***	-.609***	-.573***	-.652***	-.628***	-.805***
Scott						
Stress 1	.157	.015	.038	-.313*	-.055	-.132
Stress 2	.038	-.458***	-.409***	-.703***	-.461***	-.575***
Ellerslie						
Stress 1	-.062	.080	.379	-.214	-.179	-.178
Stress 2	-.412***	-.224	-.061	-.846***	-.617***	-.790***
Breton						
Stress 1	-.279	-.655***	-.542***	-.812***	-.773***	-.769***
Stress 2	-.486***	-.697***	-.707***	-.792***	-.666***	-.859***

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

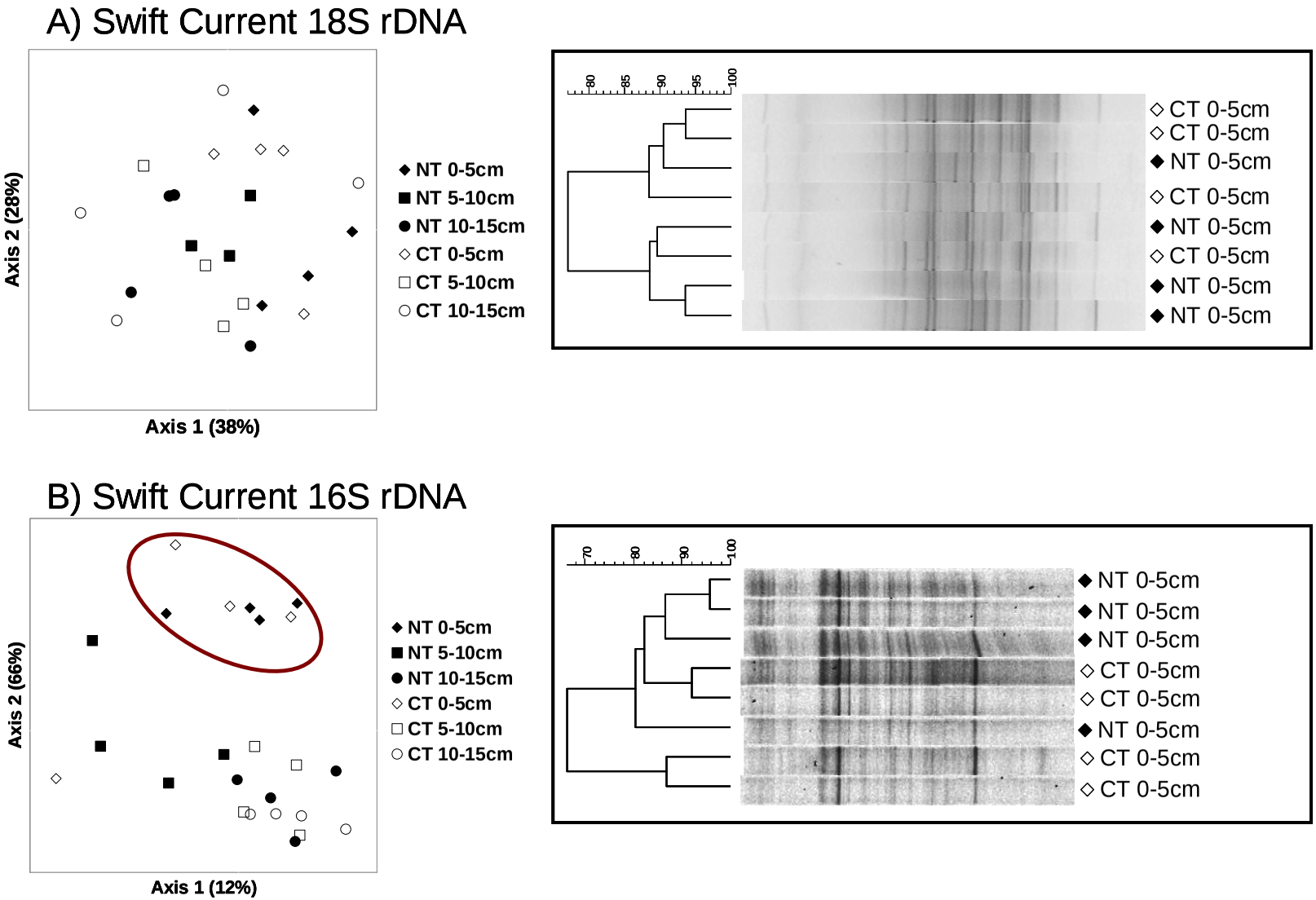

Figure 4.2 Non-metric multidimensional scaling (MDS) analysis (all depths) and dendrogram analysis (0- to 5-cm depth only) for DGGE banding patterns of A) fungal 18S rDNA (MDS final stress 24.3) and B) bacterial 16S rDNA (MDS final stress 12.7) communities in no-till (NT) and conventional-till (CT) soils at Swift Current.

which separated communities along Axis 2 and yielded a distinct grouping for surface soils (Fig. 4.2B). Similar to the ordination analysis of fungal fingerprints of the 0- to 5-cm depth soils, cluster analysis showed high variability among replicate field plots and resulted in a lack of clustering by tillage (Fig. 4.2B), which was also the case at the two lower depth increments (Fig. B.2).

PLFA analysis indicated biomass for AMF, Gr+ and Gr- bacteria was 20 to 60% greater in surface soil (0- to 5-cm) in NT than CT. In the 5- to 10-cm and 10- to 15-cm depths, biomass of these groups increased by 53% or decreased by 25% in NT compared to CT (Figs. 4.3 and 4.4). Biomass of Gr+, Gr- and AMF biomarkers was greatest at the soil surface and significantly decreased ($P<0.05$) at lower depths (Figs. 4.3 and 4.4; Table 4.2) in both NT and CT soils. The relative abundance of AMF and Gr+ bacteria was affected by tillage (Table 4.2) with greater dominance of AMF in NT soils and of Gr+ bacteria in CT soils. Relative abundance of Gr+ bacteria also increased significantly with depth. Physiological stress biomarkers were significantly greater in CT than NT soils and increased with depth (Fig. 4.5). Analysis of community structure using PLFA profiles and MDS yielded a two-dimensional solution that separated communities by depth, but not tillage along both Axes 1 and 2 (Fig. 4.6) and accounted for 98% of the variability.

4.5.1.2 Scott

Fungal 18S rDNA fingerprinting at Scott yielded a three-dimensional solution (final stress = 14.7) with the majority of variation accounted for in Axis 1 where some separation of depth occurred (Fig. 4.7A). Fungal banding patterns grouped by tillage along Axis 3, with the

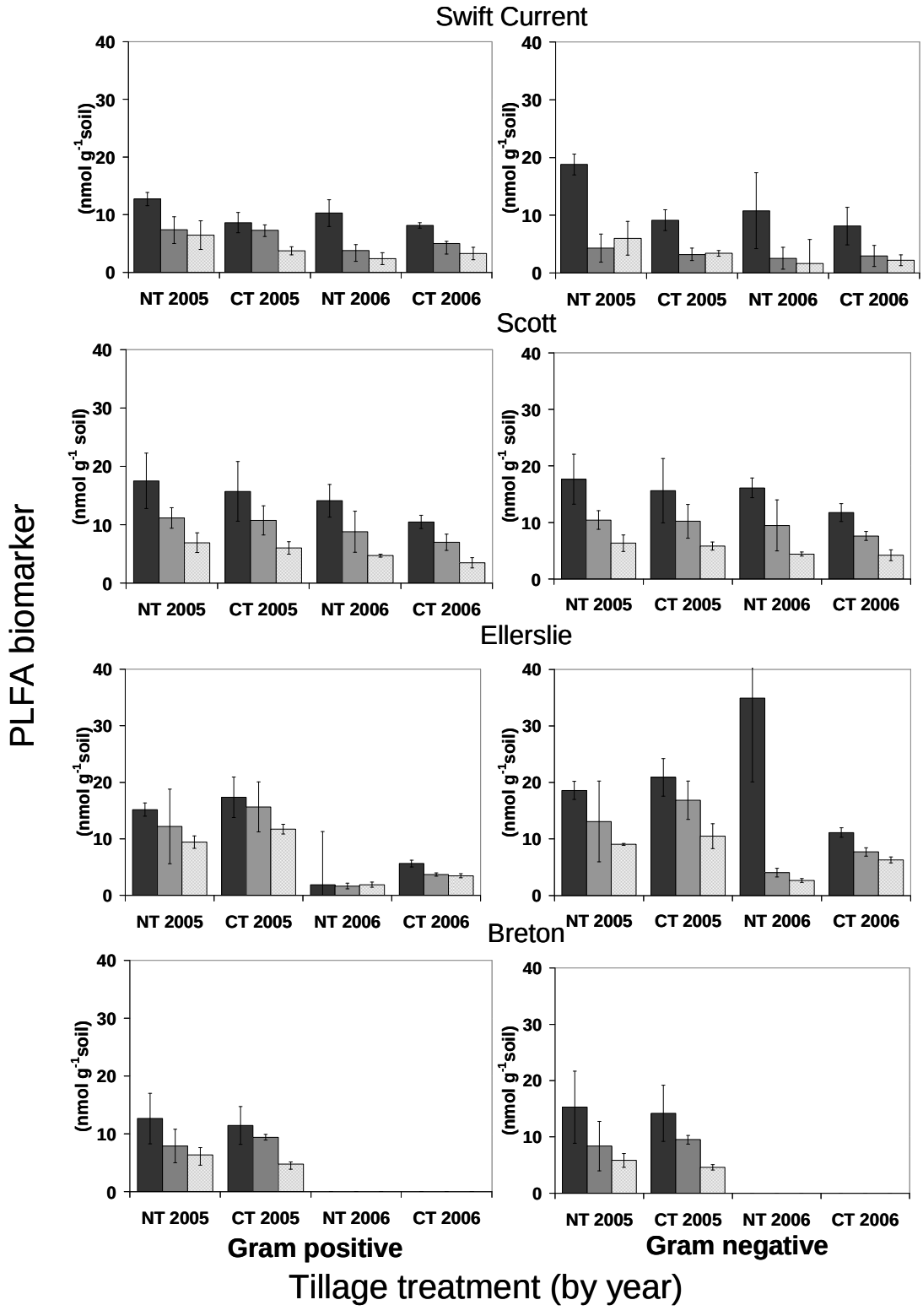

Figure 4.3 Biomass of Gr+ and Gr- bacteria measured at three depth increments in no-till (NT) and conventional-till (CT) soils at four long-term tillage sites in 2005 and 2006. Error bars represent standard deviations (n=4).

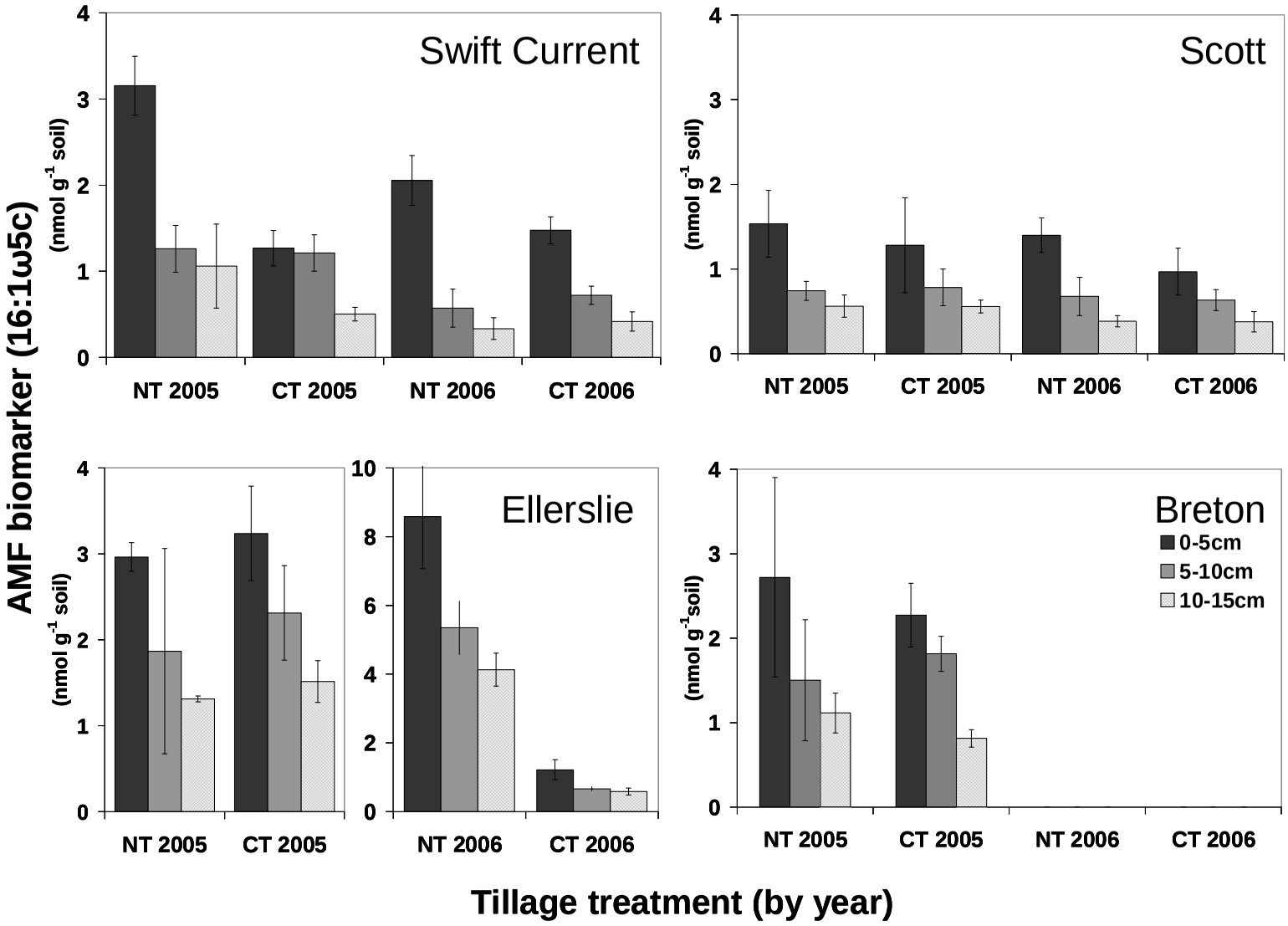

Figure 4.4 Biomass of arbuscular mycorrhizal fungi (AMF) measured at three depth increments in no-till (NT) and conventional-till (CT) soils at four long-term tillage sites in 2005 and 2006. Error bars represent standard deviations (n=4).

Table 4.2 Analysis of variance (ANOVA) for absolute and relative abundance of PLFA biomarkers in long-term no-till (NT) and conventional-till (CT) soils at Swift Current in 2005 and 2006.

	AMF		Gram positive		Gram negative	
	nmol g^{-1}	mol%	nmol g^{-1}	mol%	nmol g^{-1}	mol%
Year	***	NS	***	NS	***	NS
Tillage	***	**	**	**	***	NS
Depth	***	NS	***	***	***	**
YxT	***	NS	**	NS	***	NS
YxD	NS	NS	NS	NS	**	NS
TxD	***	NS	*	NS	***	**
YxTxD	**	NS	NS	NS	NS	NS

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

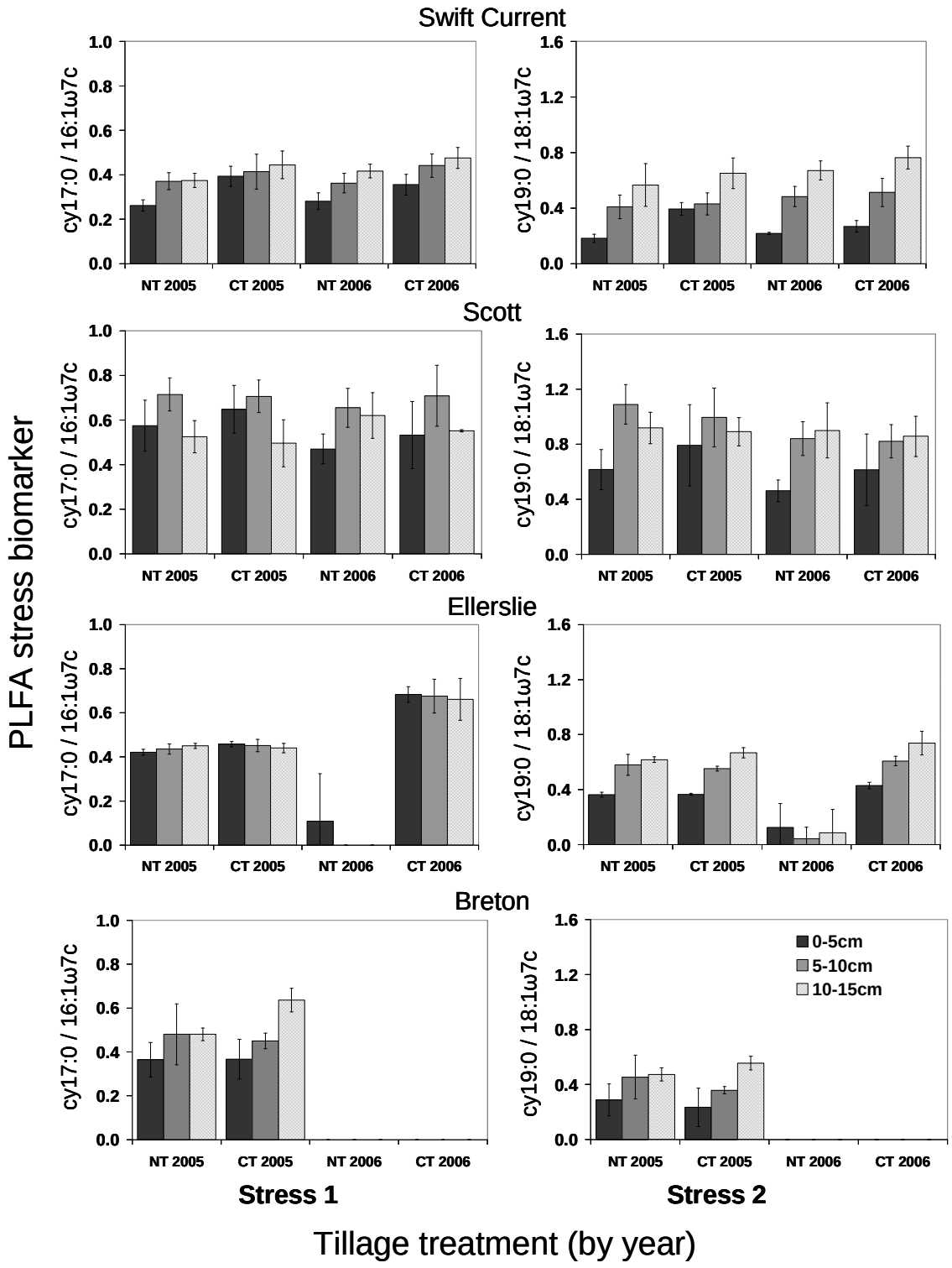

Figure 4.5 Physiological stress biomarkers measured at three depth increments in no-till (NT) and conventional-till (CT) soils at four long-term tillage sites in 2005 and 2006. Error bars represent standard deviation (n=4).

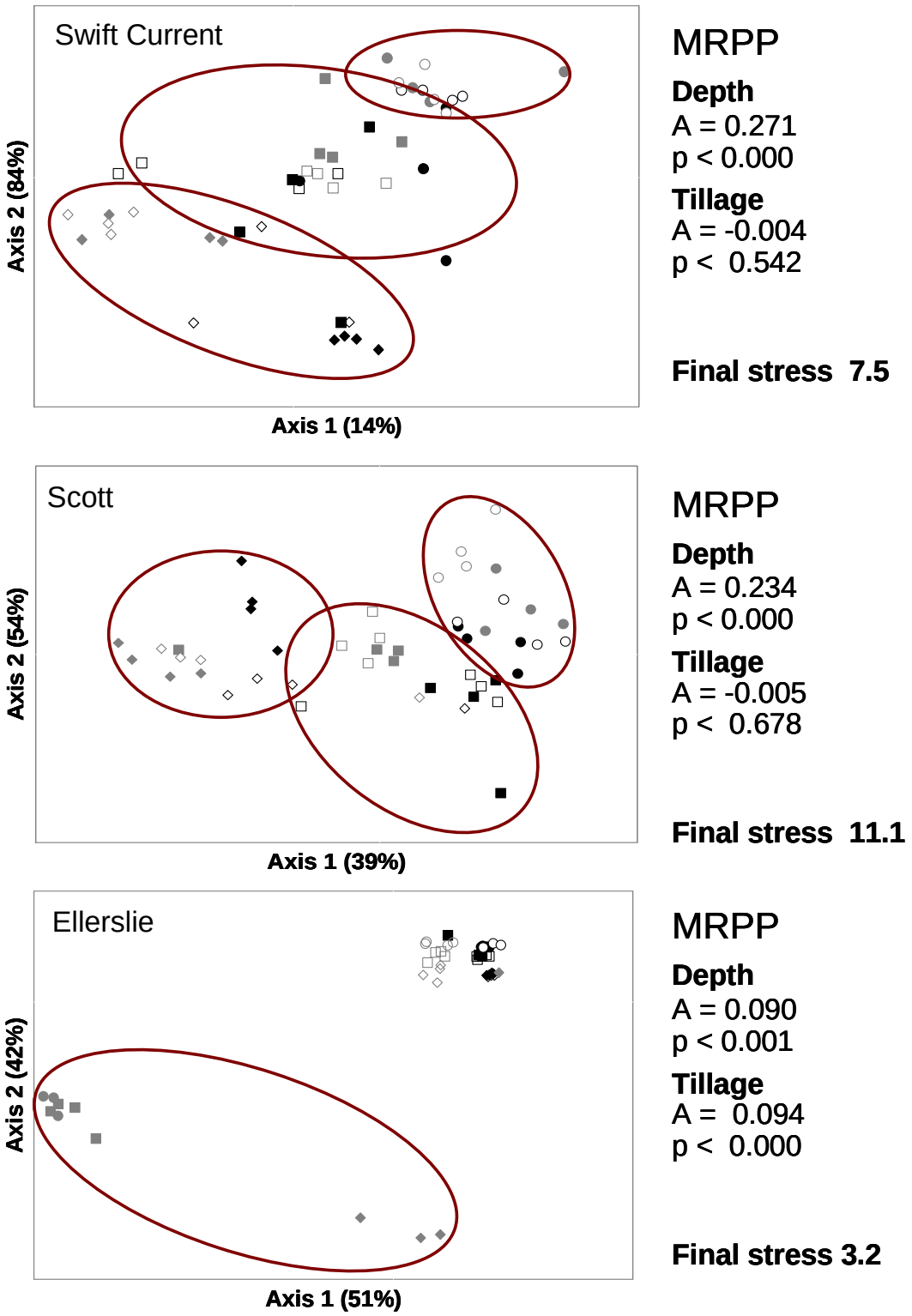

Figure 4.6 Non-metric multidimensional scaling (MDS) ordination analysis and multiple response permutation procedure (MRPP) of no-till (NT) and conventional-till (CT) PLFA profiles at the Swift Current, Scott and Ellerslie sites.

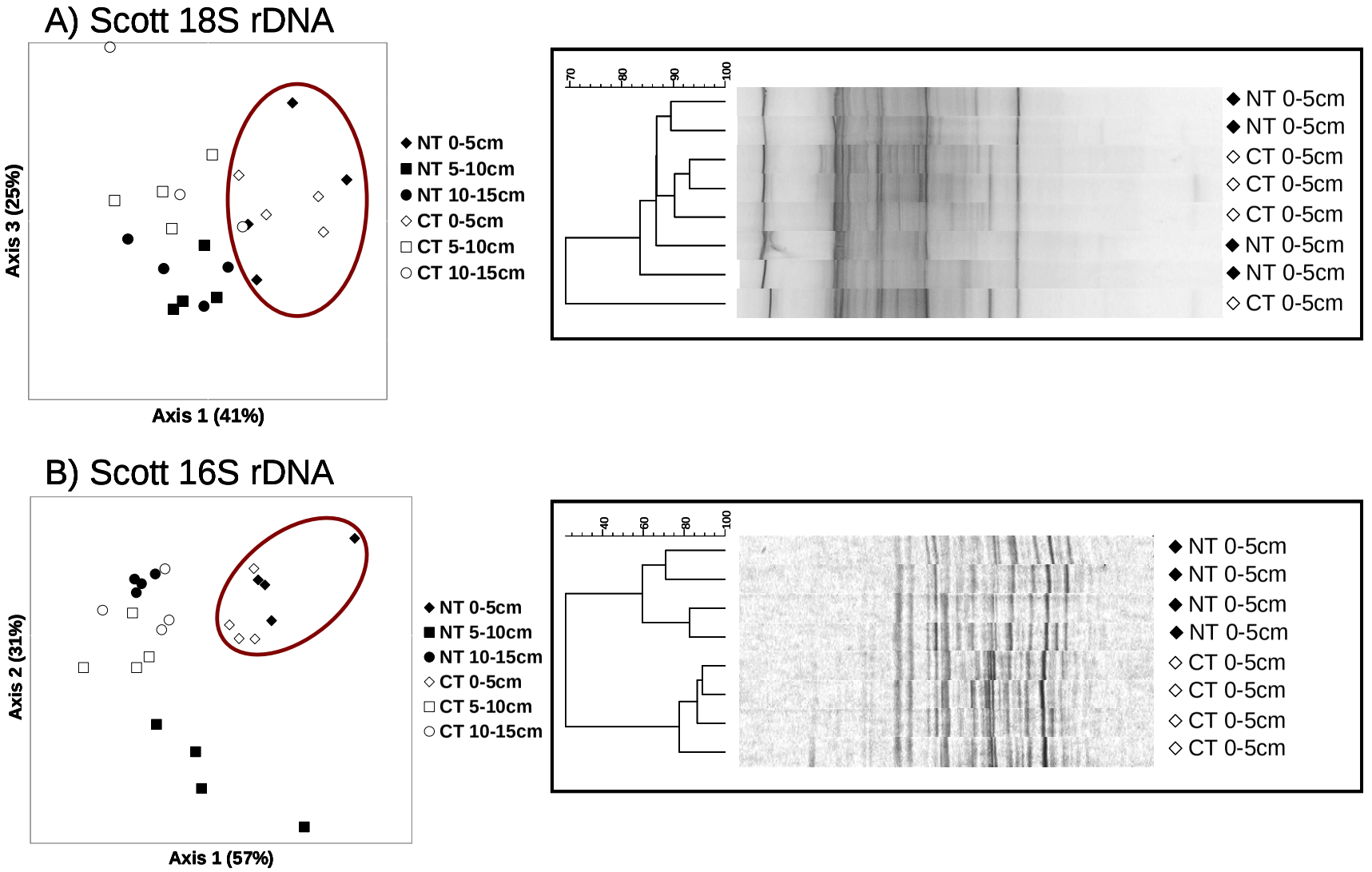

Figure 4.7 Non-metric multidimensional scaling (MDS) analysis (all depths) and dendrogram analysis (0- to 5-cm depth only) for DGGE banding patterns of A) fungal 18S rDNA (MDS final stress 14.7) and B) bacterial 16S rDNA (MDS final stress 19.0) communities in no-till (NT) and conventional-till (CT) soils at Scott.

exception of two field replicate plots at surface depth (0- to 5-cm depth). Cluster analysis of the fungal community did not differentiate between tillage treatments in the surface soils (Fig. 4.7A) or at either of the other two depth increments (Fig. B.2). Ordination of bacterial 16S genes resulted in a two-dimensional solution (final stress = 19.0) and showed a clear grouping of surface soils along Axis 1 (Fig. 4.7B). In the NT soils, there was a distinct grouping of 5- to 10-cm soils along Axis 2. Unlike cluster analysis at any of the other sites, 16S rDNA genes in the surface soils showed distinct clustering according to tillage treatment at Scott (Fig. 4.7B). No clustering of 18S rDNA or 16S rDNA genes by tillage treatment was apparent at the 5- to 10-cm and 10- to 15-cm depths (Fig. B.4).

PLFA biomarkers for microbial functional groups at Scott were less affected by tillage than at the Swift Current site. Only the Gr+ biomarkers (Fig. 4.3) showed significant differences between NT and CT and the interaction of tillage with depth was far less pronounced at this site (Table 4.3). However, there was a non-significant general trend for the abundance (mol %) of AMF and Gr+ and Gr- bacteria to be equal or slightly higher in NT across all depths. Biomass of AMF, Gr+ and Gr- bacteria did not vary significantly among the two years but decreased significantly with depth (Figs. 4.3 and 4.4). Stress biomarkers at Scott were not affected by tillage, but were greatest at the intermediate depth (Fig. 4.5). Community structure evaluated by MDS ordination yielded a two-dimensional solution (cumulatively accounting for 93% of variability) that separated microbial communities by depth along Axis 1 (Fig. 4.6).

4.5.1.3 Ellerslie

At Ellerslie, MDS analysis of fungal genes resulted in a two-dimensional solution (final stress = 12.6). A clear separation of 0- to 5-cm depth soils from lower depths, but no grouping

Table 4.3 Analysis of variance (ANOVA) for absolute and relative abundance of PLFA biomarkers in long-term no-till (NT) and conventional-till (CT) soils at Scott in 2005 and 2006.

	AMF		Gram positive		Gram negative	
	nmol g^{-1}	mol%	nmol g^{-1}	mol%	nmol g^{-1}	mol%
Year	*	NS	***	***	**	***
Tillage	NS	NS	*	NS	NS	NS
Depth	***	**	***	***	***	NS
YxT	NS	NS	NS	NS	NS	NS
YxD	NS	NS	NS	NS	NS	NS
TxD	NS	*	NS	*	NS	NS
YxTxD	NS	NS	NS	NS	NS	NS

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

according to tillage in either the surface soil (Fig. 4.8A) or at lower depths (Fig. B.5) occurred. Similarly, tillage did not differentiate communities by cluster analysis of 0-to 5-cm depth soils. Bacterial communities were similarly not dominated by the effect of tillage management. A three-dimensional solution (final stress =13.0) showed that depth in the soil profile did not affect the bacterial community structure, with the exception of surface soils in the CT treatment which grouped along Axis 2 (Fig. 4.8B). Cluster analysis of bacterial 16S rDNA genes did not show any grouping according to tillage treatment at the 0- to 5-cm (Fig. 4.8B) or 5- to 10-cm and 10- to 15-cm depth increments (Fig. B.6).

Due to an overwhelming increase in fungal biomass at Ellerslie in 2006, PLFA data from 2005 and 2006 were analyzed separately. Differences in microbial biomarkers between tillage treatments at the Ellerslie in 2005 were not significant (Table 4.4). However, there was a non-significant ($P< 0.12$ to $P<0.26$) trend of biomass being higher (8 to 22%) in CT than NT. Biomass decreased significantly with depth in most cases in both years (Figs. 4.3 and 4.4). In 2006, fungal biomass increased dramatically over other microbial groups. For example, Gr+ bacteria (Fig. 4.3) were 45 to 65% less abundant in NT than CT. Gram negative bacteria (Fig. 4.3) and AMF (Fig. 4.4) biomass was significantly greater in NT vs. CT in 2006 with a consistent increase of 85% in AMF at all depths. Stress biomarkers did not differ between tillage treatments, or among depths with the exception of the NT soil in 2006 where stress biomarkers were negligible (Fig. 4.5). Ordination analysis using MDS demonstrated a clear effect of tillage on community composition in 2006 (Fig. 4.6) which was likely driven by the effect of a large

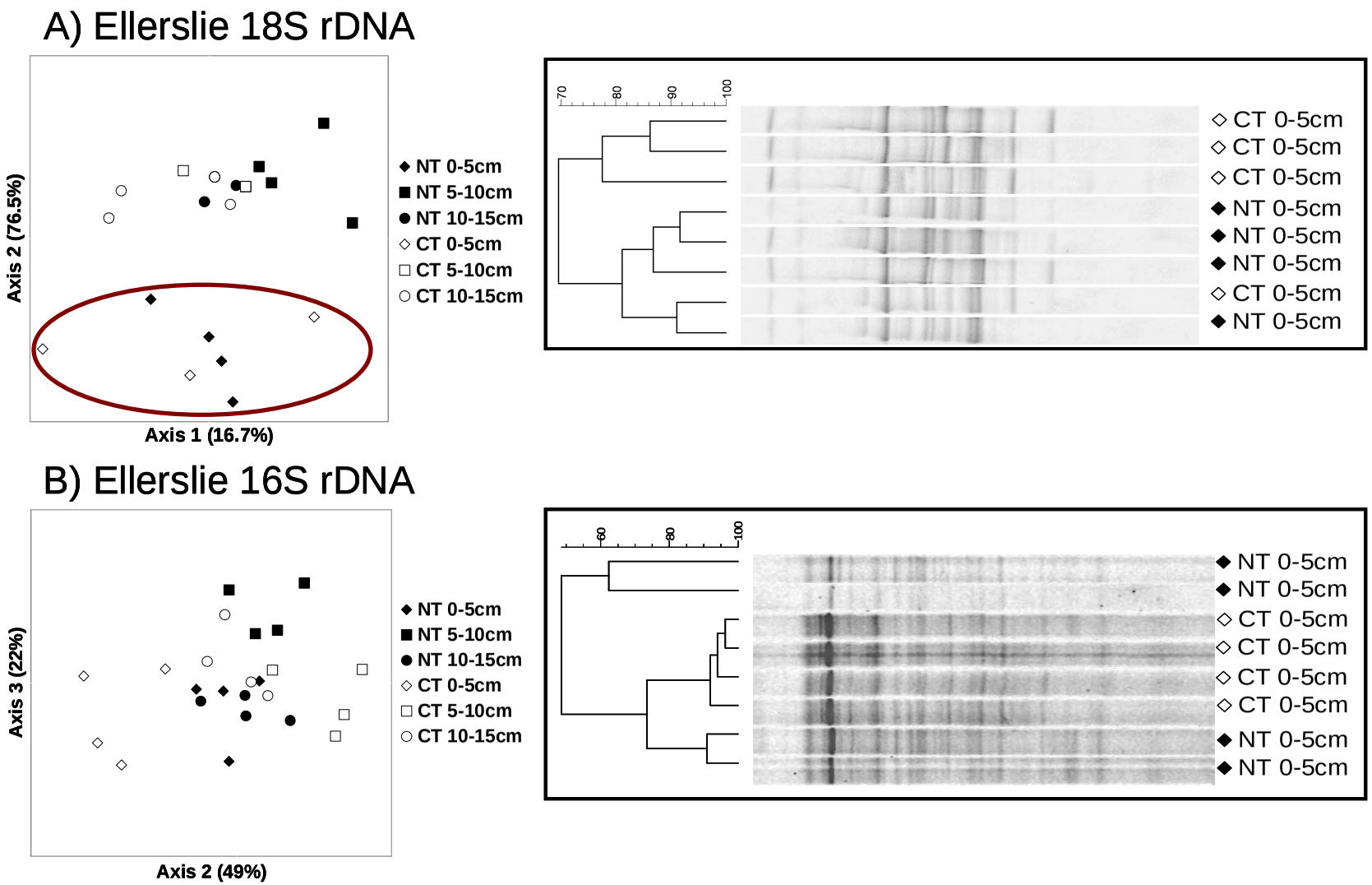

Figure 4.8 Non-metric multidimensional scaling (MDS) analysis (all depths) and dendrogram analysis (0- to 5-cm depth only) for DGGE banding patterns of A) fungal 18S rDNA (MDS final stress 12.6) and B) bacterial 16S rDNA (MDS final stress 13.0) communities in no-till (NT) and conventional-till (CT) soils at Ellerslie.

Table 4.4 Analysis of variance (ANOVA) for absolute and relative abundance of PLFA biomarker in long-term no-till (NT) and conventional-till (CT) soils at Ellerslie in 2005 and 2006.

	AMF		Gram positive		Gram negative	
	nmol g^{-1}	mol%	nmol g^{-1}	mol%	nmol g^{-1}	mol%
			2005			
Tillage	NS	NS	NS	NS	NS	NS
Depth	***	***	*	***	***	**
TxD	NS	NS	NS	NS	NS	NS
			2006			
Tillage	***	***	NS	***	*	***
Depth	***	***	NS	NS	***	***
TxD	***	***	NS	NS	***	***

*, **, *** Significant at $P \leq 0.05$, 0.01, and 0.001, respectively.

increase in fungal biomass in the NT plots. Communities were differentiated along both Axis 1 and 2 which accounted for 93% of the variability in the data.

4.5.1.4 Breton

As mentioned earlier, 2006 Breton data was not analyzed due to a crop failure in 2005 and subsequent infestation by dandelion in spring 2006 in the NT plots. These factors masked any potential effect of tillage on microbial community structure. In 2005, there was no significant effect of tillage on Gr+ bacteria, Gr- bacteria or AMF (Table 4.5), but there was a general trend in which biomass was slightly higher (7 to 27%) in NT than CT (Figs. 4.3 and 4.4). Similar to trends at other sites, microbial biomass of AMF, Gr+ and Gr- bacteria decreased significantly ($P<0.001$) with depth in 2005 (Figs. 4.3 and 4.4). The Stress 1 biomarker increased with depth, but not as a function of tillage, while a significant interaction of tillage with depth for the Stress 2 biomarker resulted from higher stress in NT for the 0- to 5-cm and 5- to 10-cm depths and higher stress in CT for the 10- to 15-cm depth (Fig. 4.6). Analysis of PLFA profiles using MDS yielded a simple one-dimensional solution that accounted for 98% of variability and differentiated soils by depth.

4.6 Discussion

In agricultural systems, and particularly in NT systems where crop residues remain on the soil surface, plant-derived substrates become vertically stratified. Given the dependence of microbial community structure on resource quality and availability, it is likely that this change in spatial resource distribution affects community structure. Accordingly, differences in microbial community structure between CT and NT using culture independent methods including PLFA

Table 4.5 Analysis of variance (ANOVA) for absolute and relative abundance of PLFA biomarker in long-term no-till (NT) and conventional-till (CT) soils at Breton in 2005.

	AMF		Gram positive		Gram negative	
	nmol g^{-1}	mol%	nmol g^{-1}	mol%	nmol g^{-1}	mol%
Tillage	NS	NS	NS	NS	NS	NS
Depth	***	NS	***	***	***	NS
TxD	NS	NS	NS	NS	NS	NS

*** Significant at $P \leq 0.001$.

and phylogenetic fingerprinting have been demonstrated (Feng, et al., 2003; Peixoto et al., 2006; Acosta-Martinez et al., 2007; Minoshima et al., 2007; White and Rice, 2009). These studies varied in the length of duration of the tillage treatments from a newly converted (1 y) to more established (6 y and 19 y) NT trials and generally, tillage induced differences in microbial community structure were only prominent near or at the soil surface. In contrast, other studies observed no shift in community structure between NT and CT soils (Petersen et al., 2002; Carpenter-Boggs et al., 2003; Spedding et al., 2004). In the current study, we found consistent increases in biomass of functional groups at the soil surface in NT, but no tillage induced shift in the microbial community structure.

Similar phylogenetic fingerprints of NT and CT soils within a given depth indicated that tillage had no effect on the genetic structure of fungal and bacterial communities. However, fingerprinting of DNA using DGGE does not necessarily provide information about changes in the abundance or activity of organisms and as such shows the broader influence of soil management on microbial communities over a longer time scale. It is possible that this common phylogeny may have been derived from a soil history that preceded the 25 yr timeframe of NT and CT management in these plots. In any case, PLFA analysis was a more sensitive method with which to assess the response of microbial communities to tillage.

Previous comparison of the total, bacterial and fungal biomass in the NT and CT soils studied at these sites showed an increase in the biomass of all three groups, but no shift in the relative abundance of fungi vs. bacteria (Ch. 3; Helgason et al., 2009). In the current study, NT resulted in greater overall microbial biomass of different functional groups near the soil surface but their relative abundance was not affected by tillage (except for the Swift Current site). Positive correlations between the abundance of biomarkers and TC and TN at three of four sites

indicates that nutrient concentrations are linked to and may be a determinant of microbial community structure, irrespective of the level of tillage disturbance. Negative correlations of the physiological stress biomarker Stress 2 with both TC and TN and abundance of various microbial group biomarkers supports this relationship. Petersen et al. (2002) found limited effects of tillage on the relationships between soil and microbiological properties and concluded that common mechanisms regulate microbial dynamics in contrasting tillage systems.

Depth as a significant determinant of microbial community structure has been noted in many soils (Bausenwein et al., 2008; Hansel et al., 2008; Wang et al., 2009b). In agroecosystems, aboveground plant biomass is deposited on the soil surface, resulting in a gradient of resource distribution with depth. In agricultural systems, tillage diminishes this stratification by redistributing plant residues throughout the plow layer. For example, Wortmann et al. (2008) found a change in community composition with depth following a one-time tillage event in long-term NT soils which they largely attributed to the redistribution of C following tillage. Diversity of functional groups can also be stratified with soil depth as a result of changing physical and chemical characteristics in the soil profile. For example, proteolytic bacterial diversity in an agricultural soil was greater near the surface due to the increased quantity and heterogeneity of substrates (Fuka et al., 2009). In a rice paddy system, varying oxic conditions with soil depth altered the community composition of ammonia oxidizing bacteria, but not that of archaea (Wang et al., 2009b).

A further explanation for the lack of tillage influence on microbial community composition in these long-term experiments may be due to continuous cropping. Manipulation of soil organisms occurs primarily through the input of substrates and soil structure (Elliot and Coleman, 1988). However, in continually cropped, diverse rotation systems, microbial

communities are bolstered by a rich spectrum of resources. This is also true for C accumulation, as was demonstrated by Liang et al. (2003) who showed that continuous cropping had a greater effect than tillage on light fraction organic C accumulation in Brown and Dark Brown Chernozemic soils in Saskatchewan. Continuous cropping as an equalizer for the long-term development of microbial community structure is supported by the work of Drijber et al. (2000) who found that communities were clearly distinct among CT vs. NT, but only during the fallow phase of a long-term wheat-fallow rotation. Spedding et al. (2004) found that crop residue removal was more influential than tillage on microbial biomass and determined that microbial biomass C and N were reduced by 61% and 96%, respectively when crop residue was removed. Acosta-Martinez et al. (2007) similarly found an increase in functional diversity in continuously cropped vs. rotations that included a fallow phase and suggest that the negative effects of fallow on the soil microbial community could not be overcome through reduced tillage disturbance. Belowground biomass (i.e. roots) may also serve to dampen the effects of nutrient stratification by depositing root exudates and dead root matter throughout the rooting zone. This may provide some explain for the greater effect of tillage on microbial communities in cropping systems that include a fallow phase.

In continuously cropped systems, and especially those with diverse rotations, the relatively similar structure of the microbial community at the beginning of the growing season may largely be a function of the long-term provision of consistent and varied substrate, despite differences in tillage management. However, the divergence of the communities could occur during the growing season, owing to differences in the physical and chemical environment created by NT vs. CT. Detection of differences due to tillage is more difficult during the growing season because of the temporally variable influences of plant growth on the microbial

community (Drijber et al., 2000; Spedding et al., 2004). In this study, experimental comparisons were biased toward greater commonality than true CT and NT systems where a change in tillage management is generally accompanied by other changes to crop rotation and herbicide use as part of a broader management system. In practice it is possible that other non-disturbance related practices of CT vs. NT management systems may contribute to increased divergence of microbial communities.

The significant effect of tillage on microbial community structure at Swift Current may be related to lower organic inputs. Crop production systems at Swift Current often experience a moisture deficit and have lower overall productivity than the other sites studied and thus have reduced substrates for microbial communities. Insufficient soil moisture likely slows decomposition rates but C inputs from crop residues are also limited and lead to lower TC and TN in these soils. Highly significant correlation between bacterial stress biomarkers and TC and TN (as well as mineral N) suggests that nutrients were limiting growth of the microbial community at Swift Current. Interestingly, AMF biomass was at Swift Current was quite high, indicating an potentially enhanced importance of these ubiquitous symbiotic fungi which are known to provide resistance to plant growth stresses. This mechanism is supported by Lupwayi et al. (2001) who found that NT exerted a stronger impact on soil microbial functioning in resource limited soils.

4.7 Conclusions

In long term no-till experiments conducted on the Canadian prairies, no consistent effect of tillage on microbial community composition across a soil and climatic gradient was found. However, depth was a strong determinant of soil community structure. Even when NT and CT

communities were compared within a single depth, there was no significant effect of tillage, except at Swift Current.

Microbial DNA fingerprinting indicated that both bacterial and fungal communities in NT vs. CT had similar phlyogenetic potential. Variability among replicated field plots was as great, or greater than, tillage induced differences. The long-term nature of the paired tillage comparisons studied here provides reassurance that NT communities are likely to be in a steady-state. Sampling in both 2005 and 2006 was useful for demonstrating that in some cases, tillage management affects microbial groups differently from year to year. Examination of PLFA biomarkers exclusively in either year would not have captured annual variation and may have lead to different conclusions.

Arbuscular mycorrhizal fungi were particularly susceptible to tillage, as indicated by increased biomass in NT soils. Comparison of AMF biomass and community structure during the growing season, in the presence of host plants, would provide further insight into the potentially enhanced role of these beneficial symbionts in NT cropping systems.

Further work is needed to relate microbial community structure and function in contrasting tillage systems. While the current work focused on disturbance-induced differences in community structure by sampling prior to seeding when other more transient influences were most likely to be minimized, NT and CT communities need to be examined during the growing season. For example, resource distribution appeared to dictate changing community structure with depth. Similarly, subtle changes in the soil physical environment in NT and CT soils could result in substantially different conditions of moisture and resource availability in the soil rooting zone affecting crop growth.

Given the complexity of 16S and 18S rDNA banding patterns and high spatial variability, the sensitivity of this technique for determining changes in community structure was limited. The use of PLFA ordination analysis proved useful in discriminating changes in community structure with depth and could be more efficiently applied to future examination of NT and CT microbial community dynamics during the growing season.

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5.0 INFLUENCE OF TILLAGE MANAGEMENT ON MICROBIAL BIOMASS AND COMMUNITY STRUCTURE IN FIELD-FORMED SOIL AGGREGATES

5.1 Preface

In the previous chapters, it was demonstrated that there was no fundamental shift in the phylogenetic potential or relative abundance of microbial functional groups in bulk soils as a function of tillage management. However, changing physical and chemical conditions with depth in the soil profile resulted in a change in the absolute and relative abundance of different microbial groups. Tillage disturbance is an important factor leading to reduced aggregation in agroecosystems, and NT management can lead to greater aggregate formation and longevity as well as changes in aggregate size distribution. Soil aggregates are both the product of, and habitat for, microbial activity in soil and can provide refuge from the spatial heterogeneity of water and nutrient availability in the broader soil environment. Soil C sequestration in NT soils has been largely attributed to physical and chemical protection in aggregates. However, tradeoffs exist in the net greenhouse gas balance in NT systems which often have greater N_2O emissions than CT soils. Understanding microbial dynamics in different aggregate size fractions is important for our understanding of nutrient dynamics, and especially C storage. This work examines the effect of tillage on the microbial community structure in field-formed aggregate size fractions to determine whether disturbance-related changes in the bulk soil result in different microbial community dynamics in NT vs. CT aggregates.

5.2 Abstract

Aggregation is important for soil functioning, providing physical protection of organic matter and microbial inhabitants. Tillage disrupts aggregates, increases wind and water erosion of soils and exposes formerly protected organic matter to decomposition and losses. Microbial biomass and community dynamics in dry-sieved aggregate size classes from long-term NT and CT soils were examined using PLFA. Bacterial, fungal, and total biomass were up to 32 % greater in NT compared to CT aggregates. Aggregate size affected microbial biomass, which was higher in the 1 to 2 mm size than in other size classes. Arbuscular mycorrhizal fungi were particularly affected by tillage disturbance with increases of 40 to 60% among aggregate size classes in NT vs. CT, but glomalin related soil protein concentration did not differ between tillage treatments or among aggregate size classes. Bacterial stress biomarkers were higher in CT than NT aggregates but were not significantly correlated TC, TN or C:N ratio, indicating that the physiological status of bacteria within aggregates is not governed by simple quantity of available resources. Ordination analysis demonstrated a shift in microbial community structure between NT and CT soils that was not detected by the relative abundance of microbial PLFA functional group biomarkers. These results demonstrated greater microbial biomass and altered microbial community structure in NT vs. CT aggregates. Further research into the processes that govern microbial community structure in NT aggregates is needed to better understand how microorganisms interact with their physical environment and ultimately affect nutrient dynamics in soil.

5.3 Introduction

Aggregation is an important facet of soil structure, providing resistance to wind and water erosion, physical protection of organic matter and microsites for microbial activity. Through these and other mechanisms, aggregates integrally affect soil organic matter turnover, nutrient cycling and sustained soil productivity. The primary mechanisms to influence soil microbial communities are through alteration of soil structure and substrate inputs (i.e., crop residues) (Elliot and Coleman, 1988). In this way, soil biological activity and the physical soil environment are related through dynamic feedback mechanisms which inextricably link these two primary foundations of soil functioning.

Tillage disturbance affects soil aggregation directly through physical disruption, and indirectly through influences on the broader biological and chemical soil environment. Macroaggregate stability is especially susceptible to agronomic practices (Six et al., 2004). Macroaggregates are stabilized by plant roots, fungal hyphae, as well as by the byproducts of microbial metabolism (Tisdall and Oades, 1982) and provide habitat for soil bacteria while spatially protecting organic matter. Soil microbial community structure and aggregate persistence are thus interdependent.

Tillage affects both the level of aggregation and the rate of aggregate turnover (Six et al., 1998). Disruption during tillage events releases particulate organic matter from macroaggregates and increases organic matter turnover (Six et al., 2000). As a result, slower macroaggregate turnover under NT has been suggested as a potential mechanism for increased C storage in NT soils (Six et al., 1999). The formation of microaggregates within macroaggregates serves to protect stabile C whereas macroaggregates are more important for the storage of labile C (Bossuyt et al., 2002; Mikha and Rice, 2004; Six et al., 2000). Beare et al. (1992) concluded that

fungi contribute more to the formation of aggregates in NT than CT soils and Six et al. (2000) proposed that stable microaggregate formation within macroaggregates is vital for C sequestration in NT soils. Microbial biomass, including that of saprophytic and AM fungi often increase under NT management. However, relatively little is known about the mechanisms of that drive aggregate turnover and protect different forms of C inputs within aggregates (Six et al. 2004; Abiven, et al. 2009).

Separation of aggregates into multiple size classes can by done in many ways (e.g., dry sieving fresh or air-dry soil as well as wet-sieving of fresh or air-dry soil). The means by which the aggregates are obtained affects the way in which aggregates are both defined and potentially linked to function in the broader soil context (Ashman et al., 2009). It is common to assess water-stable aggregates (WSA) in the study of soil C dynamics (Six et al. 1999; Wright and Hons, 2005; Yamashita, et al. 2006; Olchin, et al. 2008; White and Rice, 2009) and the bulk of recent aggregate research has employed wet sieving of air-dried aggregates. However, it is clearly demonstrated that the method of aggregate separation affects the resulting distribution of aggregate sizes as well as their chemical (Sainju, 2006) and biological (Ashman et al., 2009; Paradelo and Barral, 2009) characteristics. Sainju (2006) compared aggregate separation methods and determined that for wet and dry sieved aggregate size distribution, C and N pools were well correlated. From this they concluded that dry sieving moist soil can be used as a reliable method of separating aggregates. We chose to separate aggregates into five size classes through dry-sieving because we felt that both wet sieving and air-drying compromised the *in situ* link between the aggregates obtained and their indigenous microbial inhabitants.

Previous work in long-term NT and CT soils at Swift Current, SK demonstrated an increase in total, bacterial and fungal biomass as well as microbial functional groups (e.g. AMF).

However, phylogenetic analysis of fungal and bacterial communities in bulk soils did not differ between NT and CT at the beginning of the growing season. We hypothesized that because macroaggregates are particularly susceptible to physical disruption by tillage, subtle shifts in microbial community structure may occur among field formed aggregates in NT and CT soils. We used PLFA to evaluate the abundance and community structure of soil microorganisms across aggregate size fractions.

5.4 Materials and Methods

5.4.1 Soil Collection and aggregation isolation

Aggregates were obtained from a long-term tillage experiment at Swift Current, SK, which is set up as a randomized complete block design (n=4). The experiment was initiated in 1981 and is continuously cropped in a four-year rotation of wheat-lentil-wheat-pea. Tillage disturbance in the CT soils is relatively low, generally involving only one pre-seeding pass. Soils were sampled in the spring of 2006 (which followed a lentil crop in 2005). Samples were obtained using a hand trowel from the 0- to 10-cm depth and soil was stored on ice during transport back to the laboratory and processed. Soil was first passed through a 4 mm sieve. Field-formed aggregates were isolated manually by dry-sieving 300 g of fresh soil on a series of 4 mesh sieves (2 mm; 1 mm; 500 µm, 250 µm). Sieves were rotated 90 times at a rate of 30 rotations per minute in the following order: 30 rotations clockwise, 30 rotations counterclockwise followed by 15 rotations clockwise and 15 rotations counterclockwise. No attempt was made to separate microaggregates from within macroaggregates and the aggregate size classes chosen here primarily correspond to commonly defined macroaggregate fractions (i.e. > 250 µm) with only the smallest size fraction representing microaggregates.

5.4.2 General soil characteristics

Gravimetric moisture content was determined by measuring moisture loss from fresh soil by mass after drying at 105ºC for 48 h. Aggregate sand content (50 to 500 µm particles) was determined by laser scattering particle size analysis using a Partic LA-950 (Horiba, Inc., Kyoto, Japan). Total C and N were measured by dry combustion using a LECO CNS-2000 analyzer (LECO Instruments, Ltd., St. Joseph, MI).

5.4.3 Phospholipid fatty acid analysis

PLFA was performed using the modified method of White et al. (1979), based on the original method of Bligh and Dyer (1959). Briefly, fatty acids were extracted from 4.0g of lyophilized, ground soil. Fatty acids were separated on a solid phase extraction column (0.50g Si; Varian Inc. Mississauga, ON), neutral and phospholipids were methylated and resulting fatty acid methyl esters were analyzed using a Hewlett Packard 5890 Series II gas chromatograph with a 25m Ultra 2 column (J&W Scientific). Peaks were identified using fatty acid standards and MIDI identification software (MIDI Inc., Newark, DE) and quantified based on the addition of internal standard methyl nonadecanoate (19:0). Total biomass was calculated as the sum of all identified PLFA peaks (Zelles et al. 1992). Relative fungal and bacterial biomass in NT and CT soils was assessed using the fungal biomarker 18:2ω6,9 and the sum of 13 bacterial biomarkers (i14:0, i15:0, a15:0, i16:0, 16:1ω7c, 10Me16:0, i17:0, a17:0, cy17:0, 10Me17:0, 18:1ω7, 10Me18:0, cy19:0) (Baath and Anderson, 2003). Biomarkers used to represent Gr+ were i14:0, i15:0, a15:0, i16:0, i17:0, a17:0 (Hedrick et al., 2005). Biomarkers used for Gr- were 16:1ω7t, 16:1ω9c, 16:1ω7c, 18:1ω7c, 18:1ω9c, cy17:0, and cy19:0 (Macdonald et al., 2004). Biomass of

arbuscular mycorrhizal fungi (AMF) was evaluated using the PLFA biomarker 16:1ω5c (Olsson, 1999).

5.4.4 Glomalin extraction and quantification

Easily extractable glomalin-related soil protein (GRSP) was obtained by extracting 1.0 g of soil in 8 mL of 20 m*M* sodium citrate buffer and autoclaving for 30 minutes at 121°C. Following extraction, soil was pelleted by centrifugation at 5000 x *g* for 15 min and the volume of supernatant measured. Glomalin related soil protein was measured (n=2) using the Bradford total protein assay using bovine serum albumen as a standard (Bradford, 1976; Wright et al., 1996).

5.4.5 Statistical analysis

Analysis of variance and correlation analyses were performed using SPSS version 13.0 for Windows (SPSS Inc., 2004). Homogeneity of variance was assessed using Levine's test. Relationships between soil characteristics, GRSP and PLFA biomarkers were assessed using Pearson's correlation coefficient.

Non-metric multidimensional scaling using the Sørensen distance measure was carried out in the Autopilot Slow and Thorough analysis option in PCOrd V.5.0 (McCune and Meford, 1999). A random starting point was used for initial analysis in which stress was minimized. A starting configuration for the final ordination was then supplied from the least stress solution obtained initially. Significance testing was performed using Monte Carlo analysis. Multi-response permutation procedures was performed using the Sørensen distance measure to test for differences between *a priori* groups and was carried out in PCOrd V.5.0.

5.5 Results and Discussion

Aggregates were separated using dry-sieving (Fig. 5.1) and aggregate size distribution for NT and CT soils is listed in Table 1. Sand content of the aggregates ranged from 35 to 47% and was significantly (P<0.07) greater in NT than CT as well as different among aggregate sizes (P <0.001) (Table 5.1). Total C and TN, PLFA and glomalin data were adjusted for sand content to account for differences that result from physical sorting of non-aggregate associated sand particles (Elliott et al., 1991) and have been presented on a sand-free soil dry weight basis.

Aggregate distribution was not significantly affected by tillage, but did have a significant interaction with aggregate size class (Table 5.1). There were more macroaggregates in NT than CT, except in the 1-2 mm size class. Other studies have shown a greater proportion of water stable macroaggregates in NT. vs. CT soils (Wright and Hons, 2005; Zibilske and Bradford, 2007; Alvarez and Steinbach, 2009). Dry sieving is biased towards more macroaggregates (Sainju, 2006) because it has less force for disaggregating. Forces imposed with dry sieving are not sufficient to break large aggregates apart, exposing the microaggregates from their interior (Ashman et al., 2009) which is in keeping with the hierarchical theory of aggregate formation. No-till aggregates had significantly higher TC (P<0.06) and TN (P<0.04) than CT aggregates, but C:N ratio did not significantly differ as a function of tillage (Fig. 5.2). Earlier work demonstrated that there was no significant difference in TC or TN content between NT and CT in bulk soils (not expressed on a sand-free basis) (Ch. 3, Table 3.2). Carbon to N ratio was lower in NT bulk soils, as was the non-significant trend with NT aggregates (Fig. 5.2) which may indicate differences in C quality within NT and CT aggregates. Similarly, moisture content was

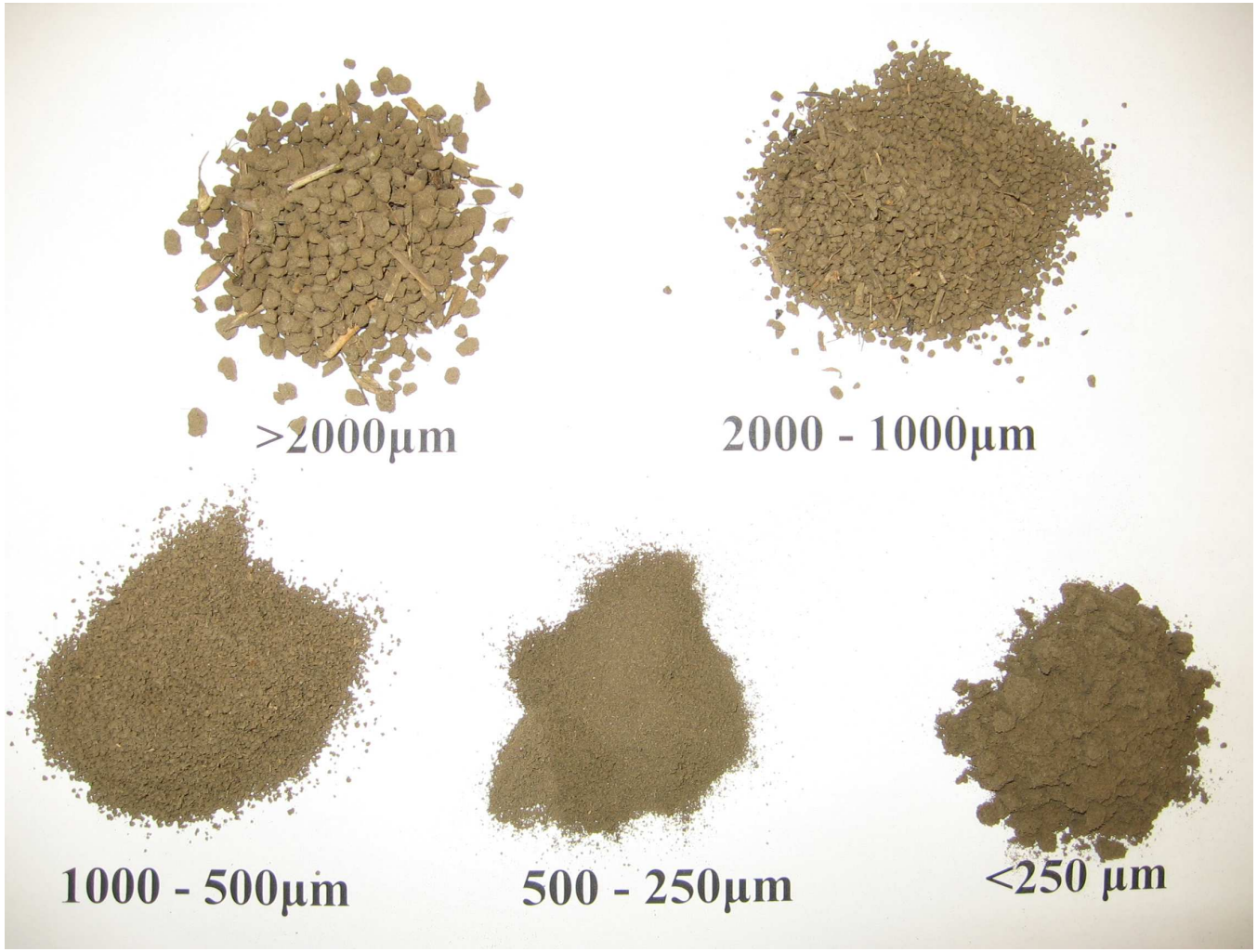

Figure 5.1 Example of dry-sieved aggregates from long-term tillage soils at Swift Current, SK.

Table 5.1 Treatment means and analysis of variance (ANOVA) for moisture content, aggregate size distribution and sand content of different no-till (NT) and conventional-till (CT) aggregates expressed as a percent of the total sample (dry weight basis).

Tillage	Aggregate size	Gravimetric moisture	Aggregate distribution	Sand content
		----------(%)----------		
NT	2 to 4 mm	11.27	36.0	38.3
CT		9.82	30.6	33.5
NT	1 to 2 mm	10.9	15.4	41.5
CT		9.8	17.5	39.5
NT	500 µm to 1 mm	11.1	20.3	36.5
CT		10.1	19.7	35.0
NT	250 to 500 µm	10.4	16.8	40.5
CT		9.9	15.5	36.5
NT	<250 µm	8.6	11.6	46.5
CT		8.3	16.7	37.8
ANOVA	Tillage (T)	*P* <0.017	*P*<0.908	*P* <0.070
	Aggregate size (A)	*P* <0.003	*P* <0.000	*P* <0.002
	TxA	*P* <0.813	*P* <0.078	*P* <0.948

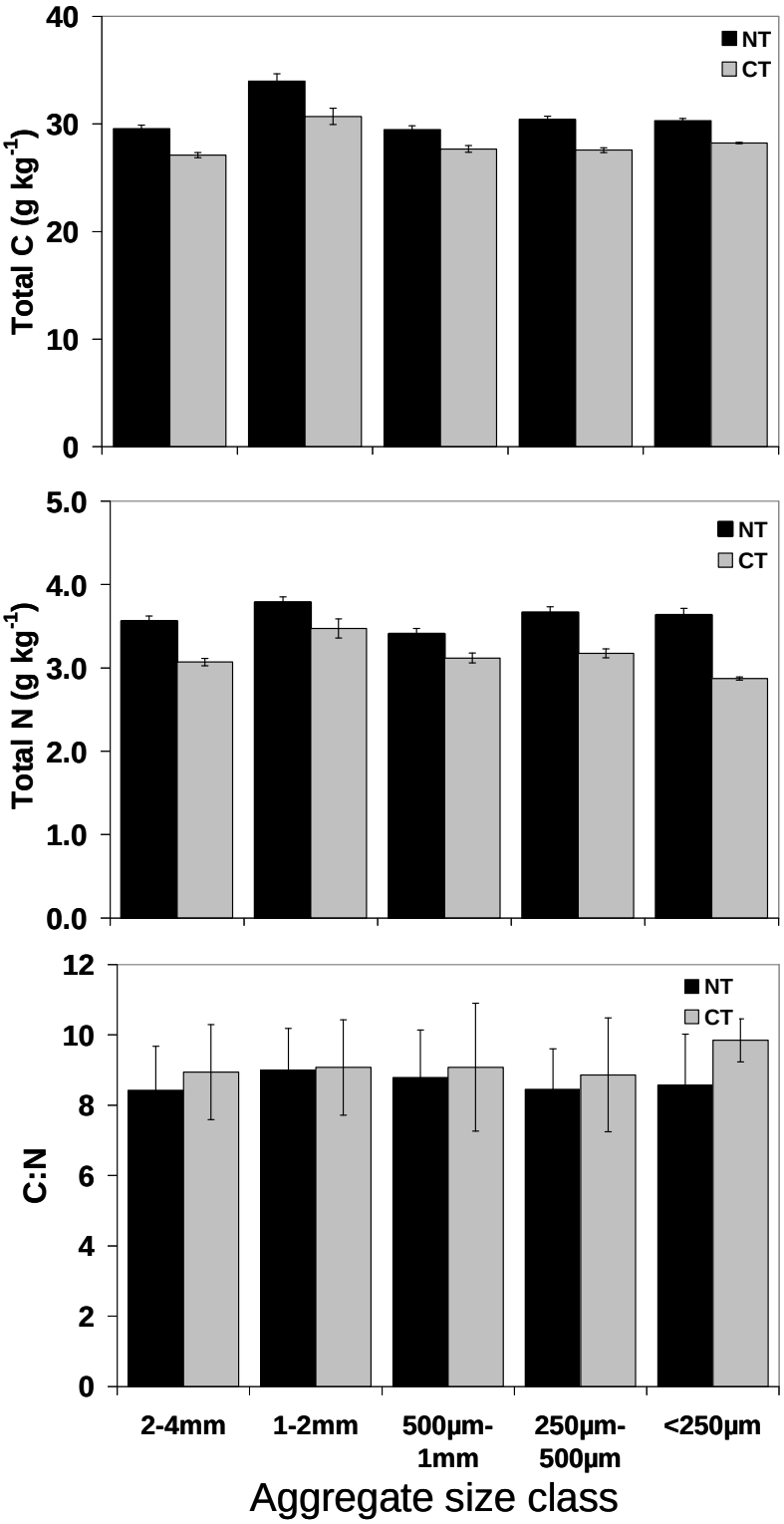

Figure 5.2 Total carbon (TC), total nitrogen (TN) and C:N ratio in dry-sieved aggregates from long-term NT and CT soils expressed on a dry soil weight basis. Error bars represent standard deviations (n=4).

greater in NT than CT aggregates (Table 5.1). There was no significant difference in TC, TN or C:N ratio among aggregate size classes.

Total, bacterial and fungal biomass were greater in NT than CT aggregates and varied significantly among different aggregate size classes (Fig. 5.3; Table 5.2). While biomass differed among tillage treatments, the relative abundance (i.e., proportion of the total community) was not different between NT and CT aggregates. Increased microbial biomass is commonly found in NT vs. CT soils (Bailey et al., 2002; Drijber et al., 2000; Feng et al., 2003; Frey et al., 1999; Minoshima et al., 2007; Spedding et al., 2004; White and Rice 2009) and was previously measured at this experimental site in the bulk surface soil (0 to 5 cm) (Ch. 3; Helgason et al., 2009). Therefore, it is not surprising to find an effect of tillage on microbial biomass in aggregates. Larger aggregates had a higher ratio of F:B biomass which is also in agreement with the hierarchal theory of aggregate formation.

Indirect evidence suggests functional microbial groups differentially inhabit and control aggregate size fractions. Simpson et al. (2004) measured greater amino sugar C in WSA of NT over CT soils, indicating that more microbial-derived C is stored in aggregates in NT soils. Guggenberger et al. (1999) found that glucosamine, a fungal-derived amino sugar accumulated in aggregates of NT soils which coincided with higher fungal biomass in the bulk soil. The current study relates fungal and bacterial biomass directly with aggregates of different sizes and shows that the F:B biomass ratio decreases with decreasing aggregate size. Fungi and roots are involved in macroaggregate formation, physically binding soil particles (Tisdall and Oades, 1982; Oades, 1984; Gupta and Germida, 1988; Chantigny et al., 1997; Bossuyt et al., 2001). Decreasing relative abundance of fungi vs. bacteria with decreasing aggregate size is consistent with this mechanism of aggregate formation. It is interesting to note that similar to the bulk soil

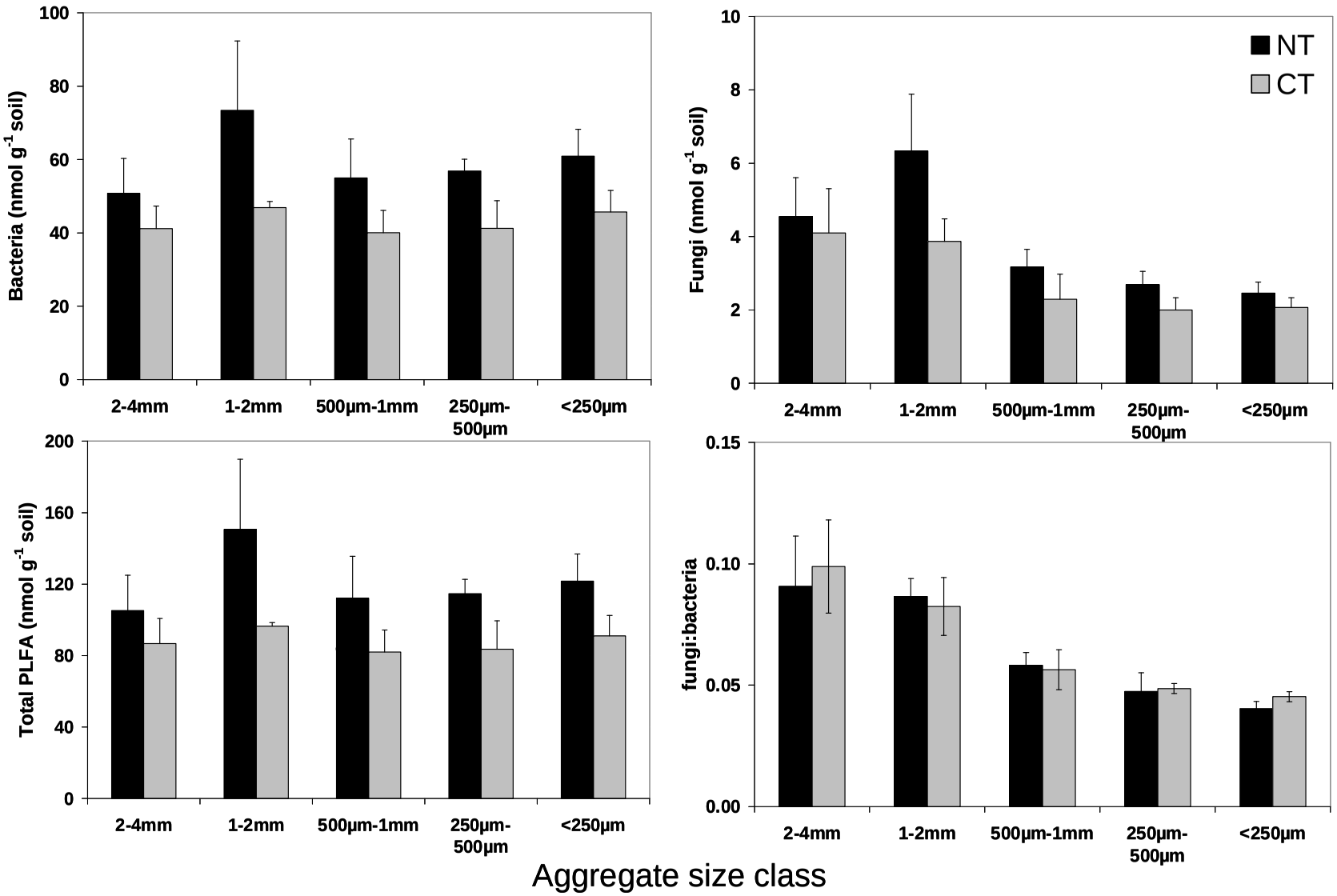

Figure 5.3 Bacterial and fungal PLFA biomarkers, total PLFA extracted and fungal:bacterial (F:B) PLFA ratio in dry-sieved aggregates from long-term no-till (NT) and conventional-till (CT) soils. Error bars represent standard deviations (n=4).

Table 5.2 Analysis of variance (ANOVA) probability values for bacterial, fungal, total and fungal:bacterial ratio (F:B) of PLFA biomarkers in dry-sieved aggregates from long-term no-till (NT) and conventional-till (CT) soils at Swift Current, Saskatchewan, Canada.

	Bacteria		Fungi		Total	F:B
	nmol g^{-1} soil	mol%	nmol g^{-1} soil	mol%	nmol g^{-1} soil	
Tillage (T)	0.000	0.318	0.001	0.722	0.000	0.628
Aggregate size (A)	0.025	0.001	0.000	0.000	0.036	0.000
TxA	0.447	0.910	0.093	0.813	0.442	0.789

(Ch. 3; Helgason et al., 2009), both fungal and bacterial biomass were greater in NT aggregates, but there was no significant difference in the relative abundance of fungi vs. bacteria. Based on the research of Simpson et al. (2004) and Guggenberger et al. (1999), it seems fungi confer greater influence on aggregate stability in NT than CT soils due to the greater overall abundance of fungal cell wall residues as a result of their recalcitrance, rather than a proportional increase in fungal biomass, at the expense of bacteria.

Similar to the total bacterial biomass, biomass (nmol g^{-1} soil) of Gr+ and Gr- bacteria was greater in NT than CT aggregates and was greatest in the 1 to 2 mm aggregate size fraction (Table 5.3). The relative abundance (mol %) of Gr+ and Gr- bacterial PLFAs extracted was not affected by either tillage or aggregate size (Table 5.3). This indicates that while both groups had a larger biomass under NT, neither became more dominant within the total community.

Saprophytic fungi and AMF produce abundant hyphe in soil and contribute to the formation of macroaggregates (Tisdall and Oades 1982). Biomass of AMF was significantly greater in NT vs. CT across all aggregate sizes (Fig. 5.4). In four out of five size classes, the AMF biomarker was 40 to 60% higher in NT than CT and in the 1-2 mm size class the AMF biomarker was 2-fold greater in NT than CT. The relative abundance of AMF vs. saprophytic fungi was greater in NT than CT and increased as aggregate size decreased (Fig. 5.5a and b). In the two largest size fractions, the ratio of AMF to fungal biomarker was 1:1, while in smaller aggregates, this ratio was >1:1.

Both saprophytic and AMF hyphae are sensitive to physical disruption by tillage (Elliot and Coleman, 1988; Wardle 1995). Decreased AMF hyphal length and inoculum potential as a result of physical soil disturbance has been clearly demonstrated (McGonigle and Millar 1996;

Table 5.3 Treatment means and Analysis of Variance (ANOVA) for absolute and relative quantity of gram positive and gram negative bacterial PLFA biomarkers in aggregates from long-term NT and CT soils.

Tillage	Aggregate size	Gram positive	Gram negative	Gram positive	Gram negative
		nmol g^{-1} soil		mol %	
NT	2-4mm	14.0	15.5	21.8	21.4
CT		11.9	14.2	20.7	24.6
NT	1-2mm	17.7	21.3	20.7	24.8
CT		12.1	14.7	20.7	25.2
NT	500µm-1mm	14.8	17.1	21.2	24.3
CT		11.2	13.2	20.9	24.7
NT	250-500µm	14.7	16.9	21.6	24.9
CT		10.7	13.5	20.4	25.6
NT	<250µm	14.0	15.8	21.4	24.4
CT		10.7	12.6	20.8	25.1
ANOVA	Tillage (T)	*P* <0.000	*P* <0.000	*P* <0.159	*P* <0.088
	Aggregate size (A)	*P* <0.043	*P* <0.015	*P* <0.754	*P* <0.397
	TxA	*P* <0.361	*P* <0.222	*P* <0.735	*P* <0.998

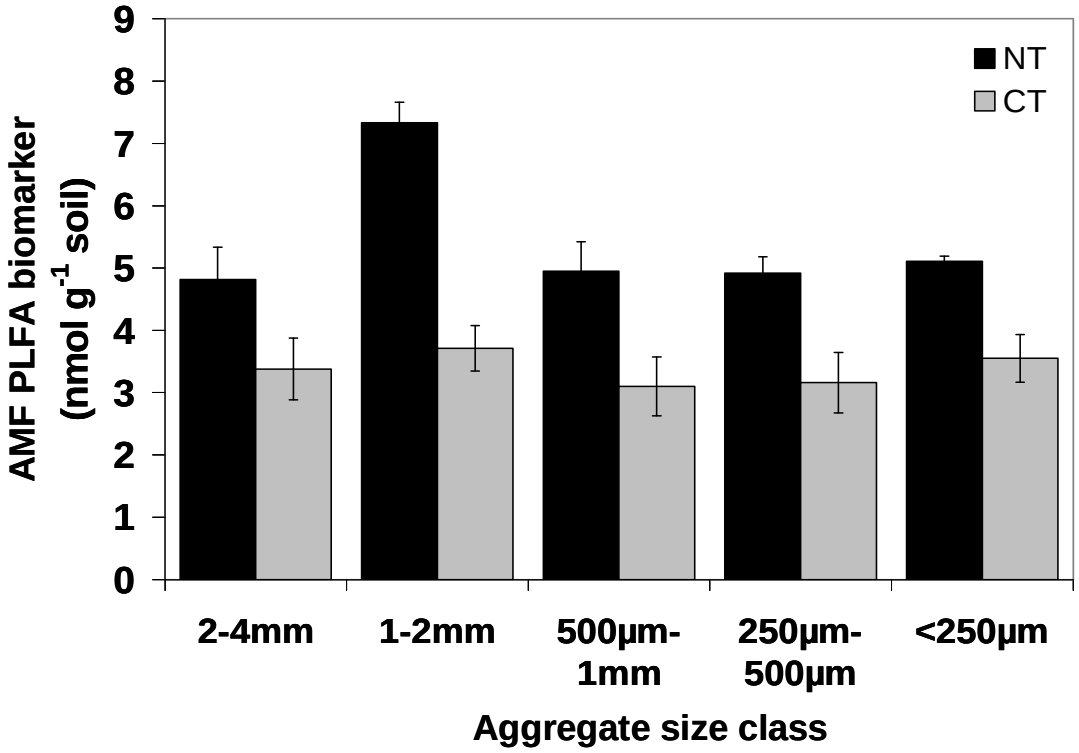

Figure 5.4 Arbuscular mycorrhizal fungi (AMF) PLFA biomarker in dry-sieved aggregates from long-term no-till (NT) and conventional-till (CT) soils. Error bars represent standard deviations (n=4).

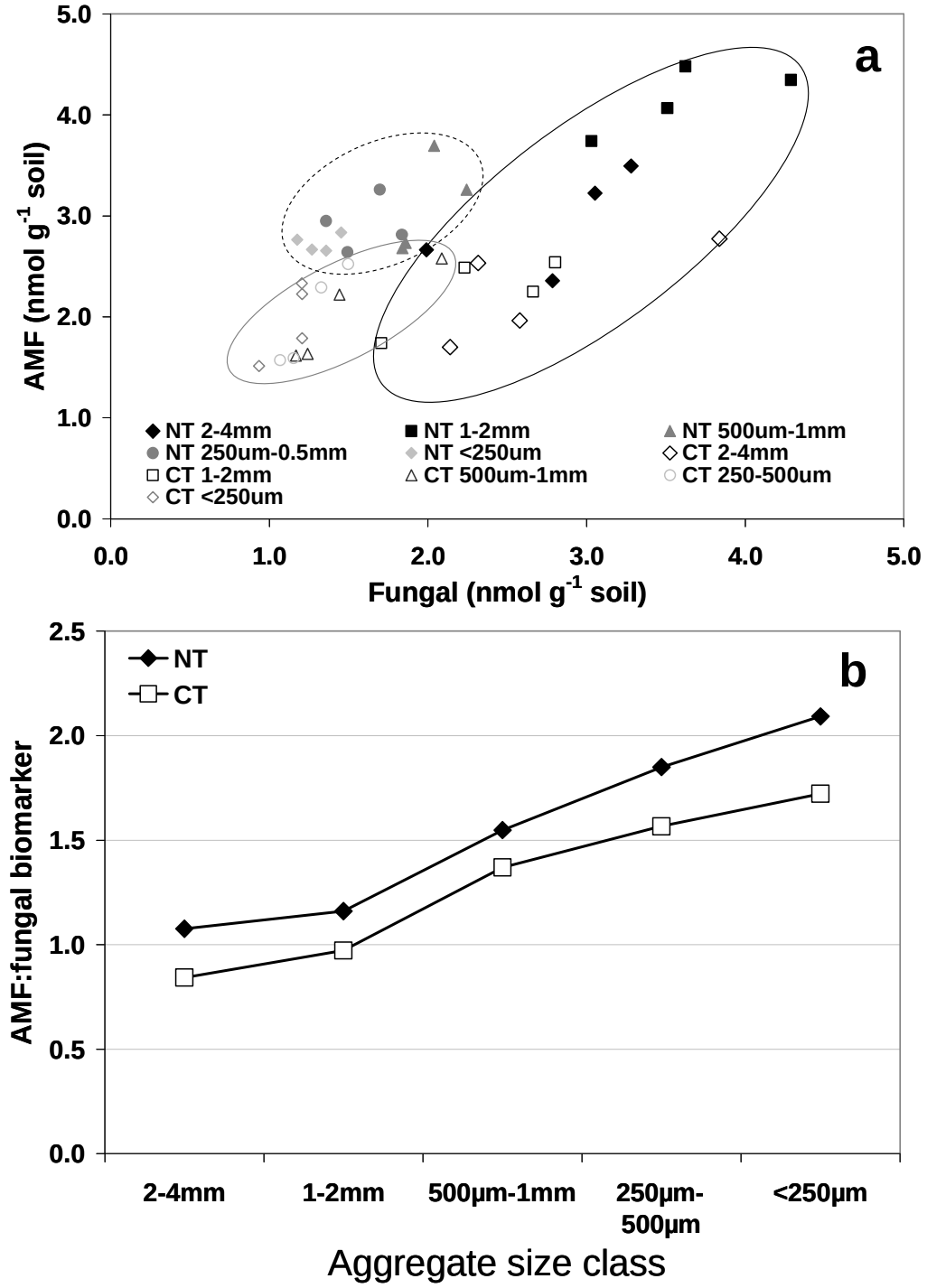

Figure 5.5 Relationship between fungal (18:2w6,9) and arbuscular mycorrhizal fungi (AMF) (16:1w5c) biomarkers (a) and the ratio of AMF:fungal biomarker (b) in dry-sieved aggregates from long-term no-till (NT) and conventional-till (CT) soils.

Kabir, et al. 1999). Wortmann et al. (2008) found a slower recovery of AMF than other microbial groups following a one time tillage event in long-term NT soils, indicating that AMF may be particularly susceptible to tillage stress. An indirect role of AMF contribution to macroaggregate formation is by promoting root growth (Bearden and Petersen, 2000). In turn, roots bind soil particles, release exudates and provide particulate organic matter that drive aggregate formation and stability (Tisdall and Oades 1982; Gale, et al., 2000; Márquez, et al., 2004; Six et al. 2004). Fungal biomass decreased with aggregate size, whereas AMF biomass remained constant among the three smallest size classes (Figs. 5.3 and 5.4) resulting in a ratio of AMF:fungi that was inversely related to aggregate size (Fig 5.5b). This suggests that AMF may be exploiting a spatial niche within small aggregates that saprophytic fungi are either less able or less apt to explore.

Glomalin, an abundant proteinaceous material in soil, coats the surfaces of hyphae and soil particles (Wright, et al. 2007) and confers water-stability to aggregates by acting as a binding agent. While the origin of glomalin remains unproven (Rillig 2004; Rosier et al. 2006), it is circumstantially linked to AMF and has a relatively long turnover time which is far slower than hyphae. A recent study by Wilson et al. (2009) found that AMF biomass was a remarkably good predictor of soil aggregation in grassland soils and in our study, was positively correlated with GRSP ($r=0.58$; $p<0.001$) (Fig. 5.6). However, easily extractable GRSP did not differ among tillage treatments or aggregate fractions (Fig. 5.7). The linear relationship between GRSP and AMF biomass was very good in CT aggregates ($r = 0.76$), but there was no relationship in NT ($r = 0.04$). Despite increased AMF biomass in NT aggregates, GRSP did not differ between tillage treatments or among aggregate size classes. In grassland soils subjected to various stresses, Wilson et al. (2009) found a continuous linear relationship between AMF biomass and aggregation (WSA) and importantly, they did not detect a threshold above which AMF

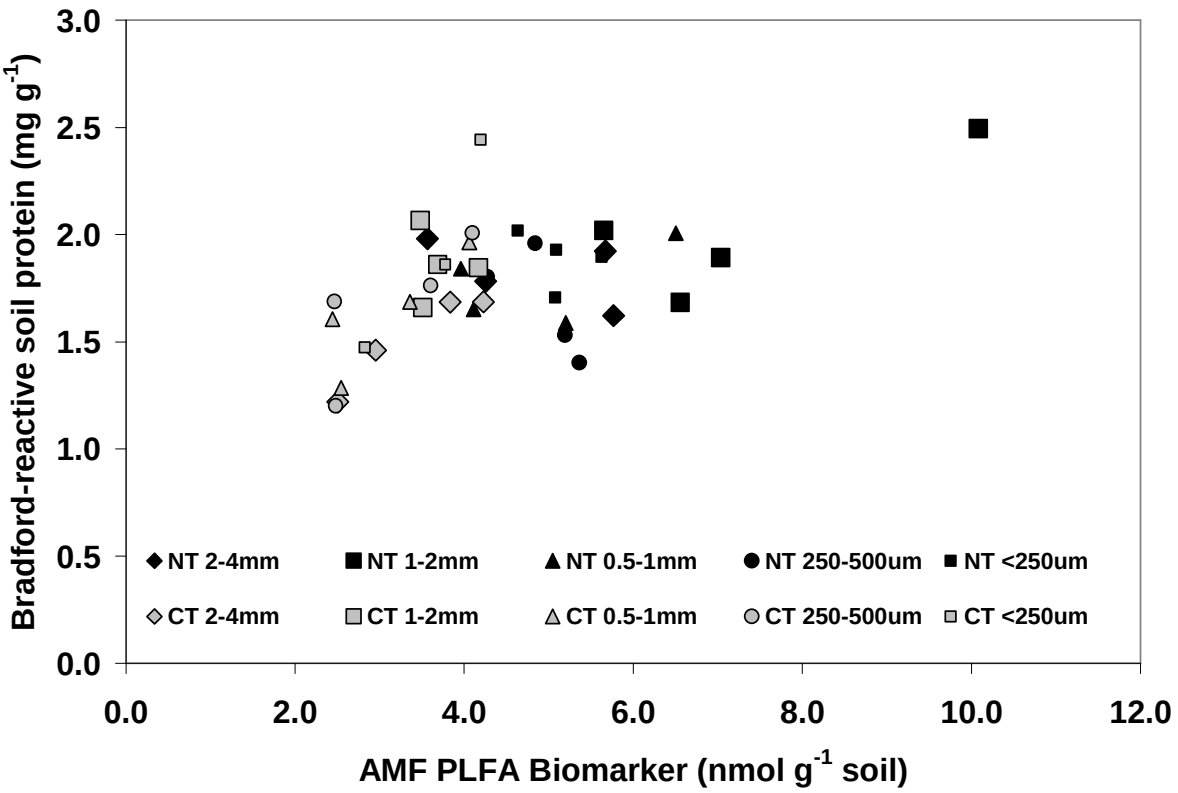

Figure 5.6 Relationship between Bradford easily extractable glomalin-related soil protein (GRSP) and arbuscular mycorrhizal fungi (AMF) biomarker in no-till (NT) and conventional-till (CT) aggregates of sizes.

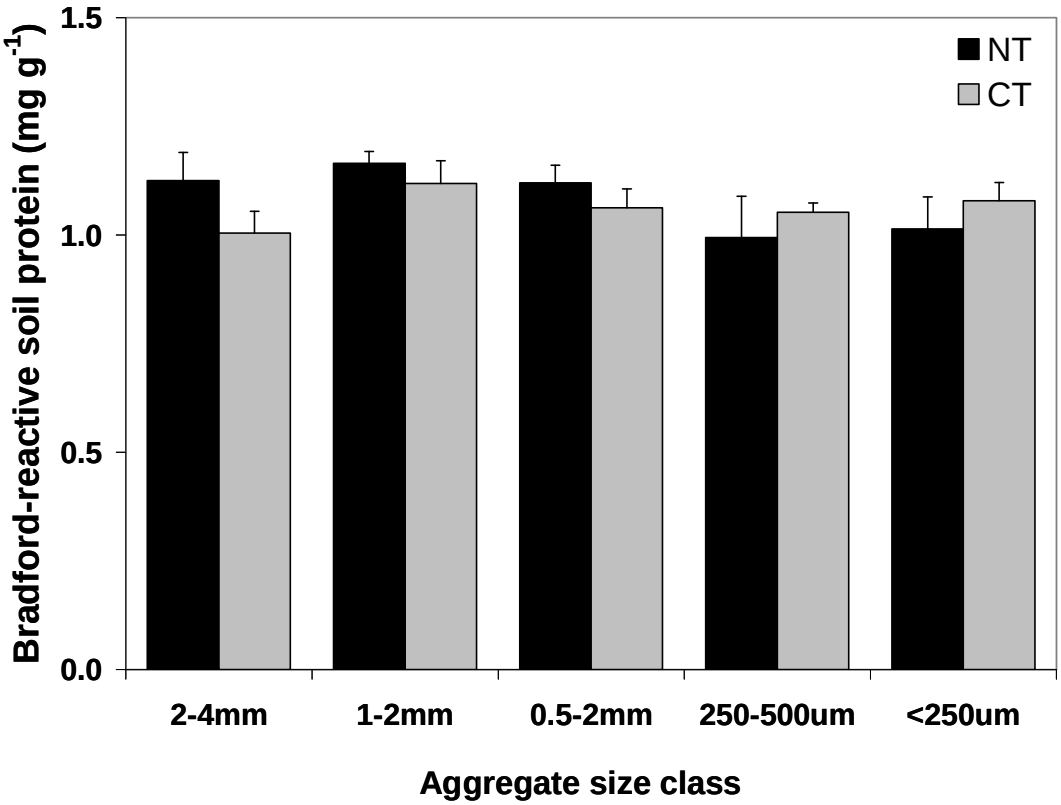

Figure 5.7 Bradford easily extractable glomalin-related soil protein (GRSP) in dry-sieved aggregates from long-term no-till (NT) and conventional-till (CT) soils. Error bars represent standard deviations (n=4).

abundance ceased to affect aggregation. The lack of positive correlation seen here in the NT aggregates could be caused by basic physiology of the AMF under different growth conditions in NT vs. CT aggregates. It is possible that soil conditions facilitate a shift in AMF physiology in NT aggregates resulting in greater production of vesicles and/or spores. Biomass of AMF was measured using the PLFA biomarker 16:1ω5c which is present in hyphae, vesicles and spores and thus an increase in biomarker concentration can occur without increased hyphal production. Because glomalin is deposited in AMF hyphal walls and on adjacent soil particles, a shift in AMF physiology could affect the ratio of PLFA biomarker to glomalin, unrelated to AMF hyphal abundance.

Wright et al. (1999) showed a strong linear relationship between GRSP and aggregate stability in soils transitioning from CT to NT and that GRSP was highly correlated with water-stable aggregation (measured in the 1 to 2 mm size class) across a wide range of soils (Wright and Upadhyaya 1998). A more recent paper (Wright et al. 2007) found that GRSP increased as water-stable aggregate size increased in NT soils, but remained constant across aggregate size classes in disturbed soils (including a CT treatment). In the present study of dry sieved aggregates, GRSP did not change significantly among aggregate size classes in either tillage treatment. Our study did not examine water-stability of aggregates; however, the lack of relationship between dry-sieved aggregate size distribution and GRSP seen here supports the theory proposed by Wright et al. (2007) that glomalin acts as a microbial glue and of Wilson et al. (2009) that AMF are instrumental in binding microaggregates together into macroaggregates.

Analysis of PLFA profiles using MDS ordination resulted in a 3-dimensional solution with a final stress of 12.0 (instability = 0.00000) accounting for 90% of the variability in the data set. Tillage management separated communities along Axis 2 and to some extent along Axis 1

(Fig. 5.8). It is interesting that ordination analysis of the entire lipid profile separated NT and CT soils, but that the relative abundance (mol %) of microbial biomarkers was not different among tillage treatments. This indicates that the tillage-induced differentiation within the community occurs at a level of organization more complex than broad groupings indicated by PLFA biomarkers. Community PLFA profiles also differed among aggregate size classes which is in contrast to the findings of Shutter and Dick (2002) who found a minimal influence of aggregate size on community FAME profiles.

Physiological stress biomarkers were significantly higher ($p<0.05$) in the CT aggregates but were not different among aggregate size classes (Fig. 5.9). These stress biomarkers were significantly correlated with CT microbial PLFA profiles and with the group biomarkers, but not with TC, TN, C:N ratio or gravimetric moisture, perhaps indicating that the type but not the overall quantity of C and /or N may be different within CT vs. NT aggregates.

Other studies of microbial communities directly associated with aggregates are few and most information regarding aggregate microbial communities is indirectly linked to the aggregates themselves by evaluation of bulk soil communities (e.g. Väisänen et al., 2005). Piexoto et al. (2006) examined 16S rRNA genes in NT vs. CT bulk soils and found that the NT community was more similar to that of an adjacent soil under forested vegetation than to the tilled soil. Due to the substantial difference in tillage intensity (the tilled soil was moldboard plowed and disk-harrowed twice per season), they attributed these differences to changes in soil structure, including increased mean weight diameter of aggregates under NT. However, Shutter and Dick (2002) showed differences in aggregate FAME profiles as a function of sampling date and winter cover cropping, indicating that lipid profiling is an effective method for determining agronomic impacts on microbial community structure in aggregates.

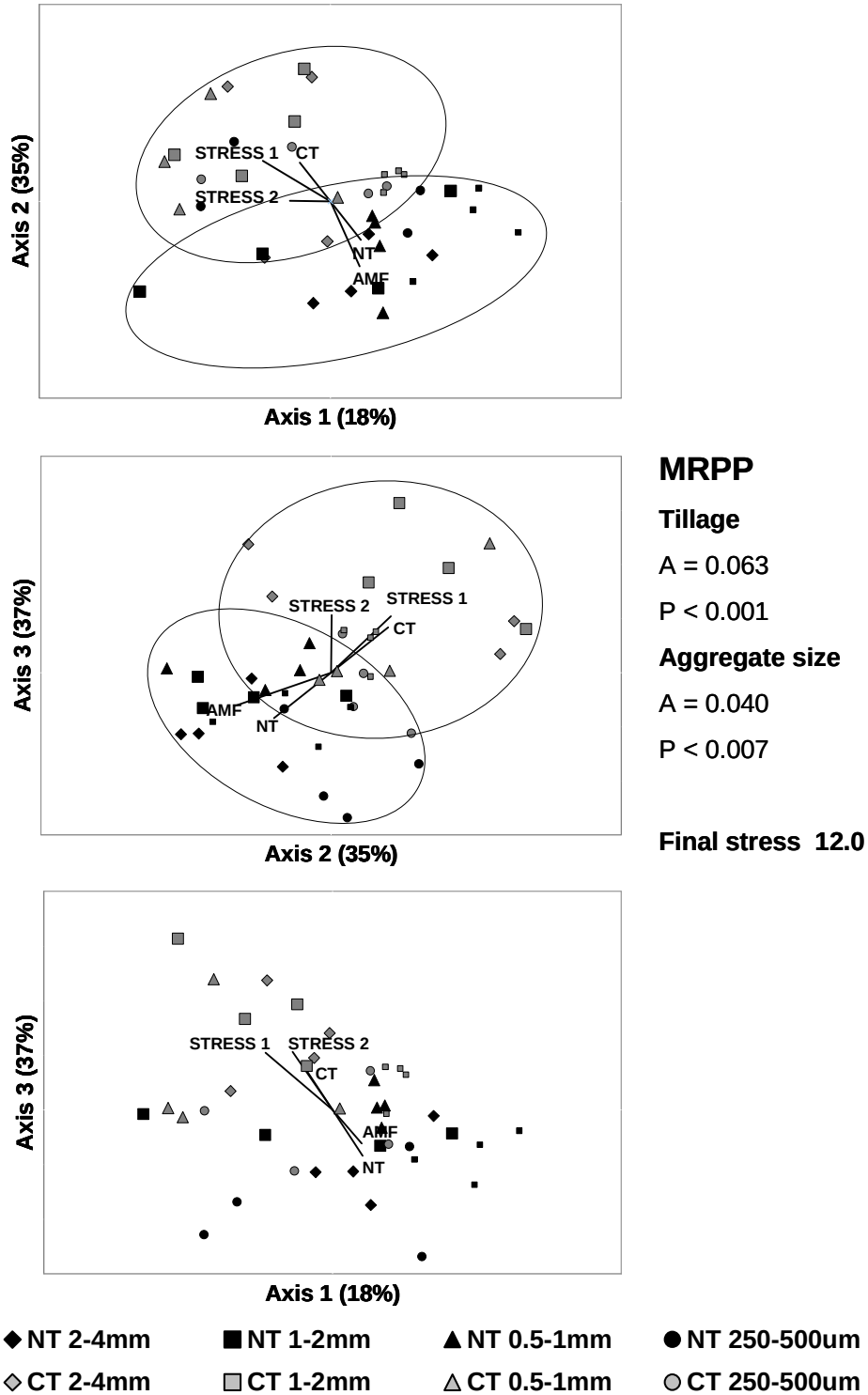

Figure 5.8 Two-dimensional depiction of the three-dimensional non-metric multidimensional scaling (MDS) ordination analysis and multiple response permutation procedure (MRPP) of PLFA profiles from dry-sieved aggregates (final stress = 12.0) in no-till (NT) and conventional-till (CT) soils.

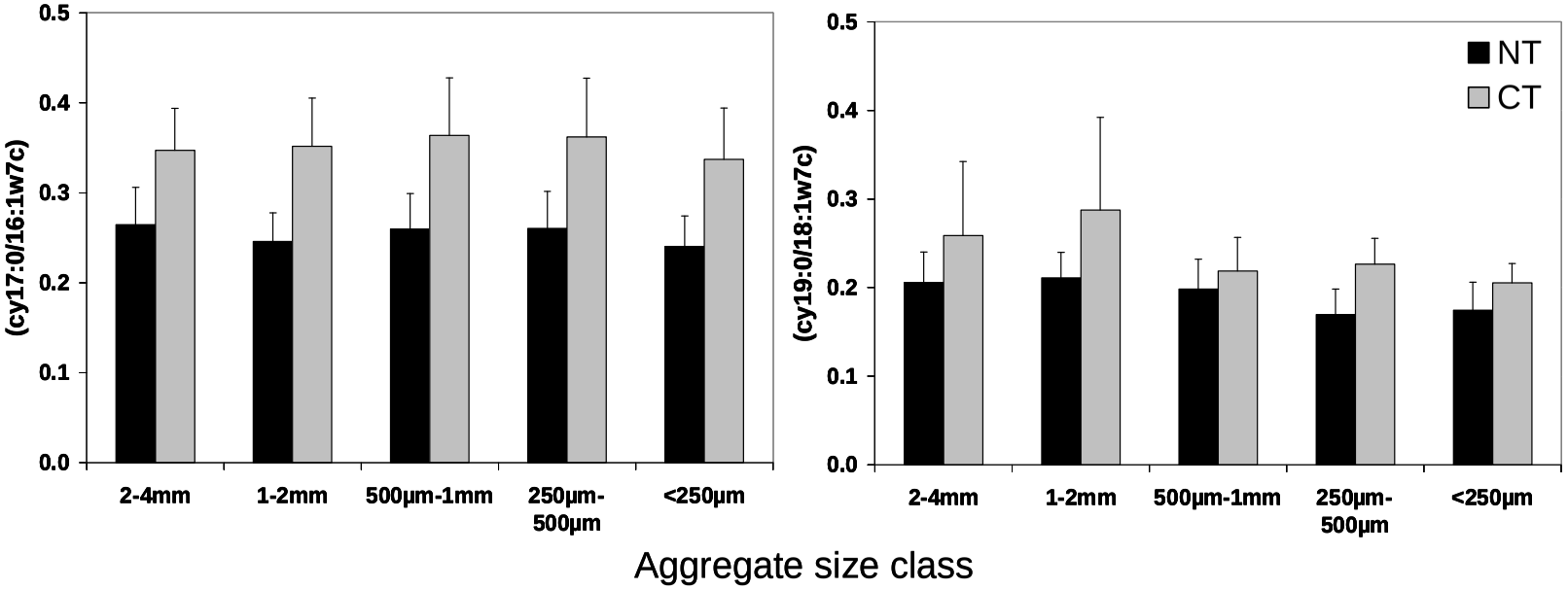

Figure 5.9 Physiological stress biomarkers Stress 1 (ratio of cy17:0 to precursor16:1ω7c) and Stress 2 (ratio of cy19:0 to precursor 18:1ω7c) in dry-sieved aggregates from long-term no-till (NT) and conventional-till (CT) soils. Error bars represent standard deviations (n=4).

5.6 Conclusions

Tillage treatments affected aggregate size distribution and associated microbial community structures in semi-arid prairie soils Swift Current, SK. Although total microbial biomass was greater in NT vs. CT aggregates, the relative abundance of microbial groups did not change. Ordination analysis of PLFA profiles showed that community structure shifted as a function of tillage at a level more specific than could be detected by group biomarkers. This indicated that responses to changing conditions under NT affected microbes at a finer scale than could be detected by group biomarkers. Biomass of AMF was greater in NT than CT soils and the proportion of AMF relative to saprophytic fungi was inversely related to aggregate size class, with AMF dominating in microaggregates. Glomalin was positively correlated with AMF in CT aggregates, but was not correlated to AMF biomass in NT aggregates. Given recent evidence of the importance of AMF for soil aggregation (e.g., Wilson et al., 2009), stabilization and C sequestration, reducing tillage disturbance to promote AMF growth and abundance is fundamental to sustainable management of agroecosystems.

Differences in C storage potential exist in NT and CT aggregates, but relatively little is understood about the mechanisms that facilitate C preservation. Further research is needed on the relationship of tillage with microbial community structure and function across aggregate size fractions.

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6.0 MINERALIZATION, NITRIFICATION AND AMMONIA OXIDIZER POPULATION SIZE IN LONG-TERM NO-TILL AND CONVENTIONALLY-TILLED SOILS

6.1 Preface

Previous chapters examined microbial community dynamics in NT and CT soils using a sampling design that maximized the capture of long-term soil disturbance effects. While no major differences in microbial community structure were found in bulk soils as a function of tillage, it was determined that depth had a significant effect on microbial community structure, likely as a result of changing resource availability in the soil profile. A more tailored examination of aggregate size fraction revealed a shift in microbial community structure within aggregates of NT vs. CT at a long-term site located at Swift Current, SK. In order to better relate this information about microbial abundance and community structure to soil processes, microbial function needs to be examined more specifically. Understanding N turnover in NT soils is necessary in order to tailor the management of N inputs to meet crop demand and minimize environmental impact. Because of the abundance of ancillary information gathered from the Swift Current long-term tillage site during other work for this dissertation, a unique opportunity existed to do some exploratory work on N turnover which lead to more specific questions about N cycling in NT soils. This study examined the processes of N mineralization and nitrification in order to gain better insight about the potential for differential N turnover in NT and CT systems.

6.2 Abstract

No-tillage is commonly practiced on the Canadian prairies with approximately 50% of annual cropland managed as such. Nitrogen availability in the soil and subsequent crop uptake and utilization often differs in NT and CT systems, but the mechanisms driving these differences are not well understood. Therefore the objective was to determine N turnover in the semi-arid Brown soil zone at Swift Current under long-term NT and CT management. Gross mineralization (GM), using the ^{15}N pool dilution method was significantly ($P<0.05$) higher in CT than NT when incubated *in situ* with rates of 0.7 and 1.0 mg N kg^{-1} d^{-1} vs. a potential GM rate of 4.0 mg N kg^{-1} d^{-1} (cores incubated at room temperature). Gross nitrification (GN) rates were extremely variable with *in situ* mean rates of 3.5 and 1.1 mg N kg^{-1} d^{-1}, and potential GN rates of 2.8 and 6.6 mg N kg^{-1} d^{-1} for NT and CT, respectively. There was no difference in potential nitrification (PN) between NT and CT soils, but PN was significantly higher in spring (17 and 18 mg N kg^{-1} d^{-1} in NT and CT, respectively) than at anthesis (13 mg N kg^{-1} d^{-1}). Abundance of *amo*A genes from ammonia oxidizing bacteria (AOB) was higher in CT than NT, but did not vary among sampling dates, while *amo*A gene abundance from ammonia oxidizing archaea (AOA) was not affected by tillage, but was higher at pre-seeding vs. anthesis. Potential nitrification was positively correlated with AOA *amo*A abundance, soil moisture and NO_3^- contents at the time of sampling, and negatively correlated with NH_4^+. These results indicate that *in situ* differences in N turnover in NT and CT soils when abiotic factors such as temperature or moisture are optimized. Further work is needed to clarify the specific effects of temperature and moisture on microbial populations involved in processes of N turnover in NT and CT soils.

6.3 Introduction

Soil tillage changes the physical soil environment and can stratify crop residue and nutrient distribution in the soil profile. Crop yield and protein content from NT and CT systems are different (McConkey et al., 1996; 2002) and the effect of tillage on N uptake varies (Soon and Arshad, 2004; Mahli et al., 2006; Malhi and Lemke, 2007). Therefore, given the wide adoption of NT on the Canadian prairies (>50% of annual cropland)(Statistics Canada 2008), understanding how tillage management affects microbial influences on N cycling and plant N availability as a function of tillage is fundamental for improving N use efficiency and crop yields.

Nitrogen turnover in soils is complex and dependent on a myriad of chemical, physical and biological interactions. These factors ultimately control N availability for crop growth and losses of N to adjacent environments. Nitrogen mineralization produces NH_4^+ from organic N and nitrification converts NH_4^+ to NO_3^-, a particularly important process in fertilized agricultural soils. These processes, along with denitrification (the conversion of NO_3^- to N gases) and biological N fixation constitute the major processes of N turnover that are relevant for crop growth and environmental losses of N from agroecosystems. Because of the increased emphasis on NT as a mitigation tool for reducing CO_2 emissions from agriculture, it is particularly important to understand nitrification dynamics in NT soils in order to create a net greenhouse gas budget which takes into account N_2O emissions resulting from nitrification and denitrification (Six et al., 2004).

The first step in the nitrification process is carried out by ammonia oxidizers which oxidize NH_4^+ to hydroxylamine (NH_2OH), a reaction catalyzed by ammonia monooxygenase. The functional gene encoding the active subunit of ammonia monooxygenase (*amo*A) can be

used to study these organisms (Rotthauwe et al., 1997; Treusch et al., 2005). Historically, ammonia oxidizing bacteria (AOB) were thought to be primarily responsible for ammonia oxidation; however, recent work has demonstrated that ammonia oxidizing archaea (AOA) *amo*A genes are also abundant in soil, outnumbering AOB *amo*A gene abundance up to 3000-fold (Leininger et al., 2006). The relative roles of ammonia oxidizing AOB and AOA in nitrification in agricultural soils remains unclear (Di et al., 2009; Jia and Conrad, 2009; Offre et al., 2009). Increased biomass near the surface in NT soils can result in increased N immobilization (Carter and Rennie, 1984; Zibilske et al., 2002) but also has the potential to increase gross N turnover rates (Booth et al., 2005). Greater overall abundance of bacteria and fungi may increase competition for NH_4^+ between heterotrophic and autotrophic organisms and could potentially decrease autotrophic nitrification rates in NT vs. CT managed soils.

The objectives of this investigation were to determine whether rates of gross mineralization (GM), gross nitrification (GN) or potential nitrification (PN) differed between long-term NT and CT soils and how AOB and AOA population size were related to nitrification potential.

6.4 Materials and Methods

6.4.1 Background information

This work was conducted in a long-term tillage experiment at Swift Current, SK. The study site was established in 1981 on an Orthic Brown Chernozemic soil. The conventional tillage treatment consisted of tillage immediately prior to seeding and basic soil characteristics at the site, as well as crop rotation sequence are listed in Table 3.1. Soils from the GM and GN assays conducted in the spring of 2008 were previously cropped to field pea in 2007. Potential

nitrification and assessment of ammonia oxidizer populations in 2009 were on wheat stubble (wheat grown in 2008) for the pre-seeding sampling date and at anthesis of the 2009 lentil crop.

6.4.2 Gross mineralization and nitrification assays

Measurement of GM and GN were measured using ^{15}N pool dilution techniques (Davidson et al., 1991; Hart et al., 1994). In the spring of 2008 (April 29), prior to seeding and cultivation in the CT plots, seven soil cores were taken from each of four replicate NT and CT plots. Cores were taken to a depth of 10 cm with a JMC Backsaver probe equipped with Zero Contamination tubes (Clements Assoc. Inc, Newton, IA) that were 2.03 cm in diameter. One core was used to determine soil NO_3^- and NH_4^+ content as well as gravimetric moisture content. Three cores from each plot were labeled using $(^{15}NH_4)SO_4$ for GM and three additional cores were labeled with $K^{15}NO_3$ for GN. Both the GM and GN assays were incubated in the field (*in situ*) as well as at room temperature in the laboratory (*ex situ*). Labeling solutions contained 30 µg N mL^{-1} at 98% ^{15}N enrichment and were added to each core in three 0.8 mL injections with an 18-guage side-port spinal needle (Davidson et al., 1991). One of the labeled cores was dismantled within 1 h of injection and extracted on-site with 2 *M* KCl. A second set of labeled cores (one each for GM and GN) from each plot was buried in the field (*in situ* assay), and a final set of cores was transported to the laboratory (*ex situ* assay) and incubated at room temperature for 24 h. Following the 24 h incubation period, the cores were dismantled and extracted with 2 *M* KCl.

Inorganic N concentration in the KCl extracts was determined colorimetrically using the SmartChem™ 200 (Westco Scientific Instruments, Brookfield, CT). Extracts were analyzed via mass spectrometry following $^{15}NH_4$ and $^{15}NO_3$ diffusion onto polytetrafluoroethylene-encased

acid traps according to a modified method of Stark and Hart (1996) as outlined in Bedard-Haughn et al. (2004). Isotope ratio of diffusion disks were determined using a RoboPrep CN elemental analyzer coupled to a continuous flow TracerMass spectrometer (Europa Scientific, SerCon Ltd., Crewe, UK. Gross mineralization and GN rates were calculated by the difference between t = 0 and t = 24 h samples as in Hart et al. (1994).

6.4.3 Potential nitrification and *amo*A gene abundance

Soil was collected from NT and CT plots on two occasions in 2009. The first sampling occurred on April 21 prior to seeding (and spring cultivation in the CT plots) and the second on July 12 during anthesis. Seven cores (3.175 cm diameter) per plot were collected to a depth of 10 cm each and bulked. Soils were stored on ice during transport back to the laboratory where they were sieved to 4 mm within 24 h. DNA was extracted from 0.50 g of field soil using the MoBio Ultra Soil DNA extraction kit (MoBio Laboratories Inc., Carlsbad, CA).

Potential nitrification was determined using the soil slurry method according to Drury et al. (2008). Briefly, 15 g of soil was weighed into 250 mL Erlenmeyer flasks and 100 mL of working solution containing 1.5 m*M* NH_4^+ and 1 m*M* PO_4^{3-} was added. Slurries were shaken at 180 rpm at room temperature and sampled at 2, 6, 20 and 24 h. At each sampling, 5 mL of slurry was pipetted into a 15 mL Falcon tube, 5 mL of 4 *M* KCl was added and inverted to mix. Samples were filtered through pre-rinsed Whatman No. 40 filter papers. Mineral N concentrations in the extracts were determined colorimetrically using the SmartChem™ 200 (Westco Scientific Instruments, Brookfield, CT). The presence of NH_4^+ was confirmed at the end of the assay to ensure that substrate for nitrification remained.

Abundance of *amo*A genes from AOB and AOA were determined using SYBR green real-time quantitative PCR. Specifically, amoA-1f/amoA-2R (Rotthauwe et al., 1997) primers were used for AOB and Arch-amoAF and Arch-amoAR (Park et al., 2006) primers for AOA. Real-time PCR was performed in a total reaction volume of 20 µL containing 1.0 µL of soil DNA extract, 10µL of QuantiTect™ SYBR® Master Mix (Qiagen, Mississaguga, Ont.), 1.0 µL of each primer and 6 µL of H_2O. Reactions were carried out in an ABI 7500 real-time PCR machine (Applied Biosystems). Amplification conditions for amoA-1F/amoA-2R were 15 min at 97°C, followed by 45 cycles of 15 s at 94°C, 40 s annealing at 60°C and 30 s at 72°C, a final step of 45 s at 78°C followed by a melt curve from 50 to 95°C. For the Arch-amoAF/Arch-amoAR PCR, conditions were 15 min at 95°C, followed by 42 cycles of 15 s at 94°C, 40 s annealing at 54°C and 30 s at 72°C, a final step of 45 s at 80°C followed by a melt curve from 50 to 95°C. Data for RT-PCR quantification were collected during the final 45 s at 78°C and 45 s at 80°C steps for AOB and AOA, respectively. Standards were created from PCR product amplified from a soil extract and purified using a GeneClean II Kit (QBiogene, Carlsbad, CA). Ten fold dilutions of the standard were made using serial dilutions in the range of 10^{-1} to 10^{-8} and were linear over four to five orders of magnitude. Real-time PCR was performed in duplicate and amplification efficiencies of 101 and 75% with r^2 values of 0.998 and 0.997 were obtained for AOB and AOA reactions, respectively. Specific amplification of the target gene was confirmed by confirmation of a single peak following melt curve analysis and on a 1% agarose gel. Quantity of DNA in the extract was determined spectrophotometrically and gene copy number reported as copy number per ng^{-1} DNA g^{-1} of dry soil. Absolute quantification was performed by comparison with the standard curves and gene copy numbers were calculated assuming 660 Da per dsDNA bp. Bacterial ammonia oxidizer population size was determined by

dividing AOB *amo*A gene copy number by 2.5 to account for multiple gene copies per chromosome (Norton et al., 2005).

6.5 Results and Discussion

Gross mineralization rate was significantly ($P<0.05$) higher in CT than NT soil in spring of 2008 (Table 6.1). Mineralization may have been limited temperature, as indicated by a significant increase ($P<0.001$) in the *ex situ* GM assay conducted using paired cores incubated at room temperature. Although it was not specifically measured, lower spring soil temperature is commonly observed in NT vs. CT soils (Gauer et al., 1982). There was no difference in GM between NT and CT soils incubated *ex situ*; thus, the difference between NT and CT soils in the *in situ* incubation may indicate an influence of initial differences in soil temperature. Incubation for 24 h at room temperature in the *ex situ* assay could have overcome this limiting factor for GM.

Gross nitrification rates were highly variable in both the *in situ* and potential assays (Table 6.1). Gross nitrification in the *in situ* assay was higher in NT than CT, while the opposite was true in the soils incubated at room temperature. For both treatments, at least one or two cores resulted in negative GN rates, indicating a violation of one or more of the assumptions of the ^{15}N pool dilution assay. One possibility is that ^{15}N-labeled NO_3^- from the labelled pool was immobilized and re-mineralized during the 24 h incubation period which would result in the negative values observed. The soils were dry in the spring of 2008 when the ^{15}N pool dilution assays were conducted, with moisture contents of 10.5 and 11.4% for NT and CT, respectively. Addition of ^{15}N-labelled solutions increased moisture content by 4 to 7%. These moisture

Table 6.1 *In situ* (incubated in the field) and *ex situ* (incubated at room temperature) gross mineralization (GM) and nitrification (GN) rates in long-term no-till (NT) and conventional-till (CT) soils at Swift Current prior to seeding in 2009.

Assay `	Tillage	Mineralization		Nitrification	
		N[†]	Mean	N	Mean
			(mg N kg^{-1} d^{-1})		(mg N kg^{-1} d^{-1})
In situ	NT	4	0.71 (0.07)[‡]	2	3.51 (4.39)
	CT	4	1.00 (0.23)	4	1.06 (0.59)
Ex situ	NT	4	3.98 (1.01)	3	2.76 (4.30)
	CT	4	3.95 (0.82)	3	6.63 (4.88)

[†] Represents the number of cores successfully analyzed.
[‡] Values in parentheses represent standard deviations.

contents resulted in water-filled pore space well below the optimal range of 40 to 60% for nitrification (Linn and Doran, 1984). Despite efforts to distribute the labelling solution evenly throughout the soil cores, it is possible that the extreme variability in GN rates is in part derived from the existence of nitrification "hot-spots" in which activity was triggered in some cores by the addition of water in the labelling solution. Soil moisture is known to affect nitrification rate (Sahrawat, 2008). Gleeson et al. (2008) found that AOB responded rapidly to re-wetting and that soil water potential influenced AOB community structure which was correlated to nitrification rate (AOA were not studied). In any case, the high variability between cores makes it difficult to determine relative treatment differences in GN rate and values listed in Table 6.1 should be interpreted with caution.

Potential nitrification was not significantly affected by tillage, but was, overall higher (P<0.001) at pre-seeding than at anthesis (Fig. 6.1). Abundance of bacterial *amo*A genes was significantly higher (P<0.06) in CT than NT soils, but was not different between sampling times (Fig. 6.2). This lack of seasonal effect was in contrast to other studies of cropped soils where population size increases over the growing season in response to N fertilizer additions (Cavagnaro et al., 2008). In the lentil phase of the crop rotation present in 2009, however, minimal N fertilizer (i.e., "starter N") was added at the time of seeding after which no additional N inputs were applied. Archaeal *amo*A gene abundance was greater in spring (P<0.001), but also did not vary as a function of tillage and was more variable among field replications (Fig. 6.2).

The abundance of AOB measured here was less than 1% of that of AOA which is in agreement with other research in fertilized agricultural soils (Leininger et al., 2006; Nicol et al., 2008; Jia and Conrad, 2009). Recent work has shown, that relative abundance of archaeal vs.

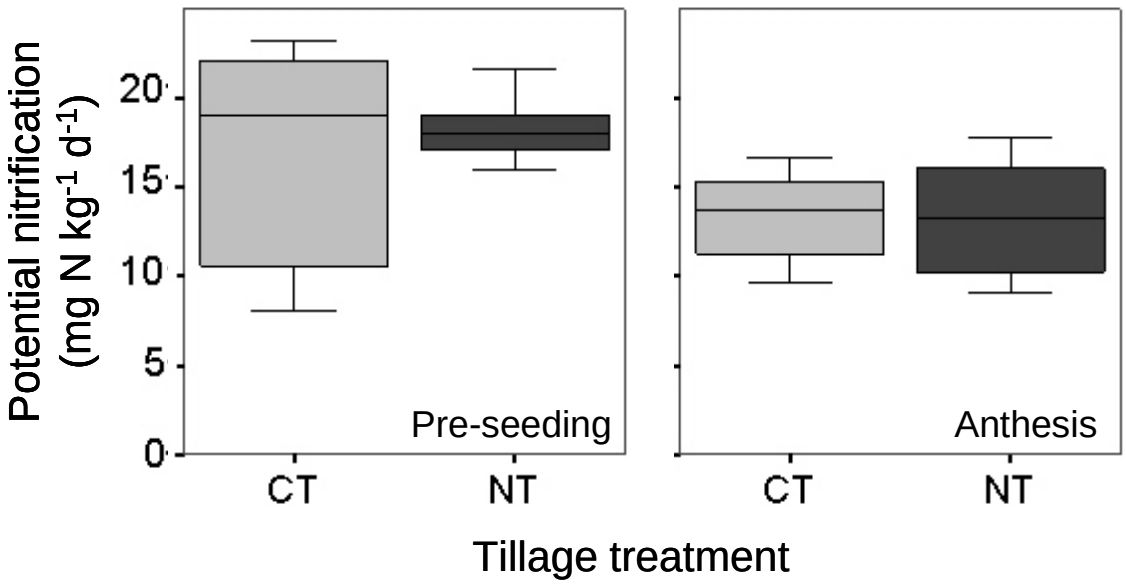

Figure 6.1 Potential nitrification (PN) rates in no-till (NT) and conventional-till (CT) soils measured prior to seeding and at anthesis in 2009.

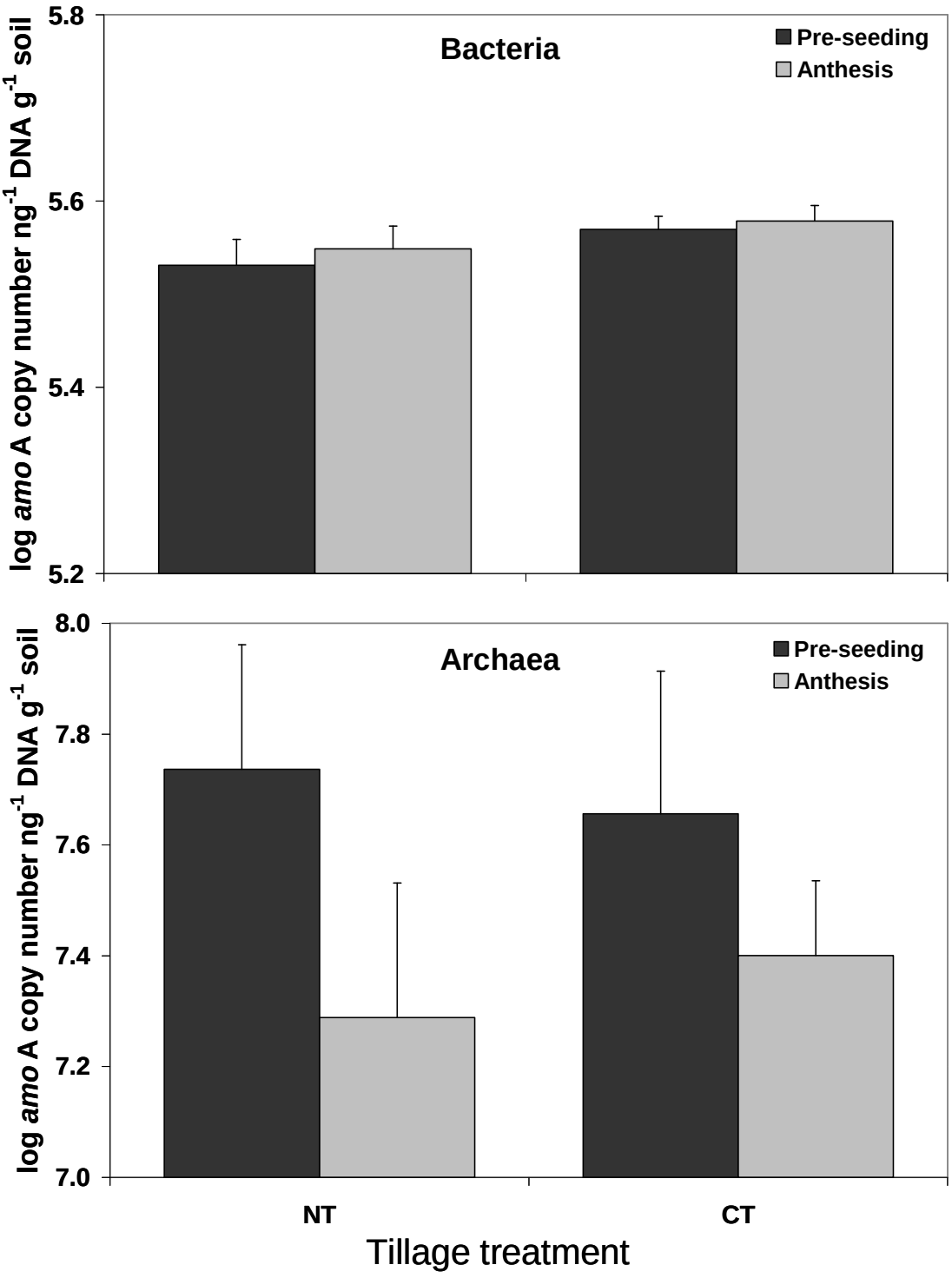

Figure 6.2 Bacterial (AOB) and archaeal (AOA) *amo*A gene abundance in no-till (NT) and conventional-till (CT) soils prior to seeding and at anthesis. Error bars represent standard deviations (n=4).

bacterial *amo*A genes does not necessarily gene expression rates of nitrification processes. In fact, AOB but not AOA *amo*A gene transcription increased concomitantly with ammonia oxidation (Di et al., 2009; Jia and Conrad, 2009). Based on this research, Di et al. (2009) and Jia and Conrad (2009) argue that bacteria and not archaea are the dominant nitrifiers in soil. In contrast, work by Offre et al. (2009) suggests that archaea were responsible for nitrification in agricultural soils. Perhaps there are site-specific relationships that drive the dominance of either AOB or AOA in nitrification processes. Schauss et al. (2009) demonstrated a soil-specific response of AOB and AOA populations with the use of the inhibitor sulfadiazine (SDZ) in manured soils. In one soil studied both AOB and AOA were inhibited by SDZ, while in a second soil only AOB were inhibited while AOA contribution to ammonia oxidation appeared to increase, indicating some functional redundancy between AOB and AOA. At this point, limited evidence in the literature seems to indicate that neither group consistently domintes all soils. More research is needed on the ecology and functioning of AOB and AOA in soils.

Potential nitrification was positively correlated to moisture content and soil NO_3^- concentration, but negatively correlated to NH_4^+ concentration which differed between sampling dates (Tables 6.2 and 6.3). The positive correlation between PN rate and soil moisture may simply reflect the natural temporal progression of soil moisture conditions during the growing season. However, it is feasible that at a moisture content of 12.5%, microbial activity in general as well as PN may have been limited. Soil NH_4^+ concentrations observed in July were much higher than those measured prior to seeding which indicates that substrate limitation in the background population was not responsible for the lower PN. An investigation of the effect of moisture on mineralization and nitrification processes is required to determine at what threshold moisture becomes limiting for these processes in the semi-arid prairie soils that are well

Table 6.2 Mean (n=4) background levels of NO_3^-, NH_4^+ and gravimetric moisture in no-till (NT) and conventional-till (CT) soils at the time of sampling for potential nitrification (PN) assays.

	April 21, 2009 (pre-seeding)			July 12, 2009 (anthesis)		
	NO_3^-	NH_4^+	Moisture	NO_3^-	NH_4^+	Moisture
	--------mg N kg^{-1}--------		%	--------mg N kg^{-1}--------		%
NT	9.8 (0.6)[†]	0.49 (0.10)	16.0 (0.6)	3.4 (1.7)	5.8 (0.4)	12.4 (0.4)
CT	8.5 (2.8)	0.71 (0.09)	15.0 (0.5)	4.5 (0.9)	4.9 (0.2)	12.5 (0.3)

[†]numbers in parentheses represent standard deviations.

Table 6.3 Pearson correlation coefficients between potential nitrification (PN) rate, soil moisture content, NO_3^- and NH_4^+ concentrations and bacterial (AOB) and archaeal (AOA) *amo*A gene abundance.

	Potential nitrification	Soil moisture	NO_3^-	NH_4^+	AOB *amo*A	AOA *amo*A
	(mg N kg^{-1} d^{-1})	(%)	--------(mg g^{-1})--------		(gene copies ng^{-1} DNA g^{-1} soil)	
Potential nitrification	1.00					
Gravimetric Moisture	0.56*	1.00				
NO_3^-	0.69**	0.89**	1.00			
NH_4^+	-0.58*	-0.94**	-0.86**	1.00		
AOB *amo*A	-0.20	-0.33	-0.18	0.18	1.00	
AOA *amo*A	0.61*	0.68**	0.83**	-0.68**	0.08	1.00

*, ** Significant at $P \leq 0.05$ and 0.01, respectively.

conditioned to circumstances of limiting moisture.

The positive correlation between PN rate and AOA *amo*A gene abundance and the corresponding lack of correlation with AOB *amo*A gene abundance circumstantially links potential nitrification to AOA (Table 6.3; Fig. 6.3). However, PN does not necessarily reflect *in situ* nitrification, nor does the presence of *amo*A genes necessarily reflect gene transcription.

Soil conditions at both samplings correlated with PN, suggesting that the microbial capacity for PN changes temporally in response to soil conditions. However, despite greater soil moisture in NT than CT in April ($P<0.05$) and greater NH_4^+ in NT than CT in July ($P<0.001$), there was no significant difference between PN of NT and CT soils at either date. Perhaps there were more broadly limiting factors at these sampling dates which were not related to the relative differences in soil conditions in NT and CT soils (e.g., substrate may have been limiting in April, while moisture was limiting in July). However, PN is measured under optimal temperature and moisture conditions, so if nitrifier communities in NT and CT soils were similar at each sampling time, then no difference in PN would result. Further investigation of the response of both AOA and AOB is warranted in order to link transcription of *amo*A genes to nitrification. Assessment of *in situ* rates of gross and net nitrification during the growing season using ^{15}N pool dilution would be more useful in linking nitrification with specific ammonia oxidizers.

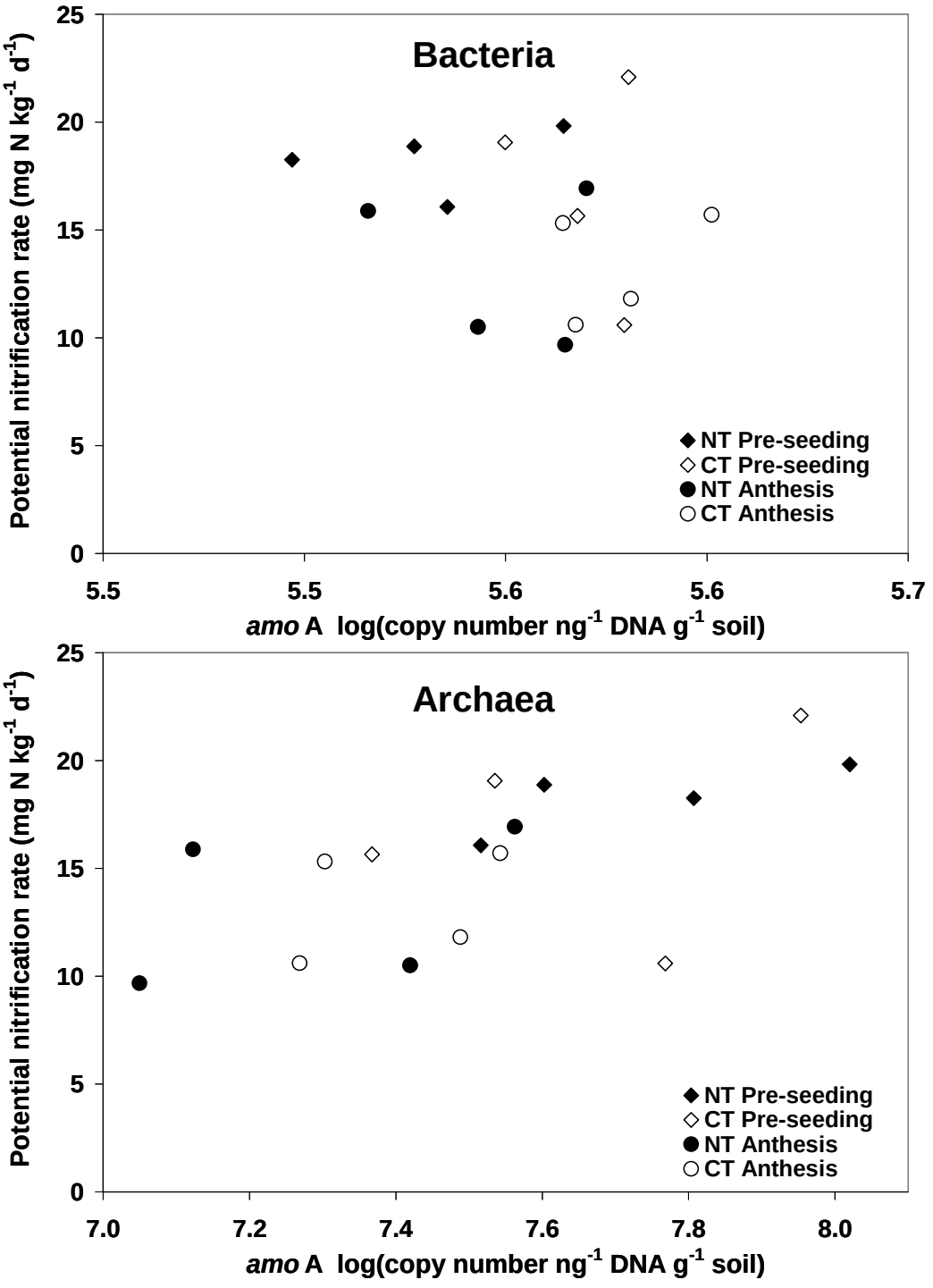

Figure 6.3 Potential nitrification rate and bacterial (AOB) and archaeal (AOA) *amo*A gene copy numbers measured in NT and CT soils prior to seeding and at anthesis in 2009.

6.6 Conclusions

This work demonstrated that in the spring, prior to seeding and N fertilization, GM was higher in CT than NT soils. However, when the same soils were incubated *ex situ* (removing temperature as a potential limitation to mineralization), there was no difference in GM between NT and CT. This indicated that substrate may not limit early season gross N mineralization in these soils and further study of gross rates of N turnover in NT soils is warranted. Differences in PN among sampling dates (pre-seeding vs. anthesis), but not between NT and CT further supports the theory that soil conditions, rather than differences in underlying nitrifier community structure dictate nitrification rates in these soils. Correlation between AOA but not AOB population size may indicate a functional role of archaea for nitrification in these soils, but further research is needed to directly relate *in situ* nitrification with AOA and AOB abundance and *amo*A gene transcription.

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7.0 SUMMARY AND CONCLUSIONS

Management of Canadian prairie agricultural soils using NT has been widely adopted in recent years. Many resources have been invested in understanding the effect of decreased soil disturbance on agronomic factors and environmental impacts of NT. No-tillage management has many beneficial attributes, particularly in reducing soil erosion and promoting organic matter accumulation, as well as reducing labour and fuel requirements for producers. However, there are a number of issues involved in implementation of NT, including reduced availability of N for crop growth following conversion from a more intensively tilled management system as well as increased weed pressure. In some cases, there may be a need to periodically till NT soils to optimize crop yields. Therefore, understanding microbial community dynamics in relation to tillage disturbance and long-term effects of NT is important to optimize productivity of NT systems and reduce risk.

Studies of bacterial and fungal dynamics in NT vs. CT systems were first conducted nearly 20 years ago, published by Beare et al. (1992) who found greater fungal dominance in NT. A particular study by Frey et al. (1999) conducted in the Great Plains is frequently sighted as evidence of fungal dominance in NT systems, and is commonly used as the basis for extrapolation of the implications of fungal diversity and function in agroecosystems, and in NT soils specifically. Many tillage studies have been conducted in recent years in divergent environments with various underlying degrees of physical disturbance (in CT soils) or only under short-term NT. Not surprisingly, results have been quite variable with respect to relative biomass and community composition among these published studies, due to the complex and variable biological, chemical and physical nature of soils in a combination of different climates

and cropping systems. In any case, a common theme of increased total microbial biomass near the soil surface in NT has emerged.

The objective of this study was to minimize confounding environmental and management factors involved in the study of microbial biomass and community structure in NT by evaluating microbial communities in long-term, continuously cropped paired NT and CT experimental plots across a climatic gradient. Using paired treatment comparisons that had been managed in NT and CT for more than 20 y allowed us to study microbial communities after the NT soils had reached steady-state. This consistency of approach provided some insight with respect to the annual influence of cropping system conditions on biomass dynamics and showed that even F:B can reverse on an year to year basis, and more importantly that F:B did not consistently shift toward a greater fungal dominance of microbial biomass.

No-till management exacerbates the accumulation of crop residues near the soil surface. However, despite the potential for enhanced nutrient stratification in NT soils and an increase in microbial biomass at the soil surface, a fundamental shift was not observed in the microbial community structure in bulk soils. Instead, the fundamental composition of the microbial communities in long-term NT and CT soils was comparable, within a given soil depth. Differences in microbial biomass and relative abundance in NT and CT were more temporary (i.e. annually), seemingly as a result of the interaction of crop rotation with environmental conditions. The significant shift in community structure among different soil depths points to the potential importance of aboveground biomass as a substrate for microbial communities.

Examination of aggregate size fractions at Swift Current demonstrated that biomass increased in NT versus CT and among different aggregate size classes. Arbuscular mycorrhizal fungi were particularly susceptible to tillage disturbance with reduced absolute and relative

abundance in CT vs. NT aggregates of all sizes. Glomalin-related soil protein, thought to confer water-stability to aggregates, did not change as a function of tillage or aggregate size class, despite the fact that GRSP is thought to be derived from AMF. Ordination analysis of PLFA profiles showed a distinct grouping of NT and CT soils, indicating that soil aggregate-associated microbial communities differed as a function of tillage, despite the lack of a change in relative abundance of microbial group biomarkers. Closer examination of organic matter chemistry and physicochemical conditions within aggregates is needed elucidate the causative mechanisms of shifts in microbial community structure in NT aggregates.

Early season GM was greater in the field for CT than NT soils, however, no difference in GM existed in the cores incubated *ex-situ*. Potential nitrification rates did not differ as a function of tillage management, but were higher prior to seeding than mid-season during peak crop flowering. Population size of AOB was greater in CT than NT, but was not correlated with potential nitrification, while AOA population was not different between NT and CT, but was correlated with PN. These results suggest that tillage-induced changes in the soil environment (e.g., moisture and temperature) may have been responsible for differences in N turnover.

The general conclusions of this dissertation are that while tillage affected the biomass of fungi and bacteria, the relative abundance of different functional groups was not different in NT and CT soils. Microbial community structure, as assessed by PLFA profiles and DNA fingerprinting, changed with depth in the soil profile indicating that nutrient stratification played a significant role in community composition. A shift in community structure within NT aggregates indicated a scaling factor of tillage, highlighting the importance of aggregates as microhabitats for microorganisms. This shift in community structure that was detectable in

aggregates, but not in bulk soils, may at least partly explain functional differences in nutrient turnover processes in NT vs. CT soils.

Future Research Directions:

Further investigation of the role of AMF and GRSP, as well as the nature of their relationship to one another, is of particular interest in NT soils due to the strong negative pressure exerted on AMF by physical tillage disturbance. It would also be of interest to examine the effect of temporary tillage disturbance in long-term NT managed soils. If glomalin is recalcitrant, as indicated in the scientific literature, then perhaps it can mitigate the loss of C under such situations. However, my research on long-term NT soils found that AMF abundance increased, but glomalin did not. A better understanding of AMF-glomalin interactions, and the nature of glomalin itself is needed to evaluate the possibility that AMF might play a special role in the sustainable management of agricultural soils through NT.

Additional study of the relationship of microbial functional groups and N turnover processes in long-term NT and CT soils is also warranted. This work found that mineralization rate prior to seeding was higher in CT than NT, but when soil were incubated at room temperature (*ex situ)*, mineralization rates were equal in CT and NT. Preliminary observation of ammonia oxidizer populations indicated that potential nitrification was not linked to AOB population size which was higher in CT than NT, but may be related to AOA. Given the current dispute in the literature regarding the relative role of AOB and AOA for nitrification in soil, it would be useful to examine this relationship in a broader range of fertilized agricultural soils to determine if the role of certain nitrifiers changes regionally, or is governed by broader factors.

Long-term field trials have special value for elucidating microbial drivers of nutrient turnover and storage. Given the ubiquitous and highly redundant nature of the microbial community in most soils, the use of long-term field trials provides a valuable “steady-state” context in which to probe these communities for information about the impacts of contrasting agronomic practices. In addition, the use of soil obtained from long-term field experiments in controlled-environment studies has the special utility of stable baseline microbial communities that have had the opportunity to adapt to different agronomic practices *in situ*. These communities can be specifically investigated for their response to targeted manipulations that are derived from either existing or anticipated agroecosystem adaptation requirements.

APPENDIX A

Field Site Descriptive Information

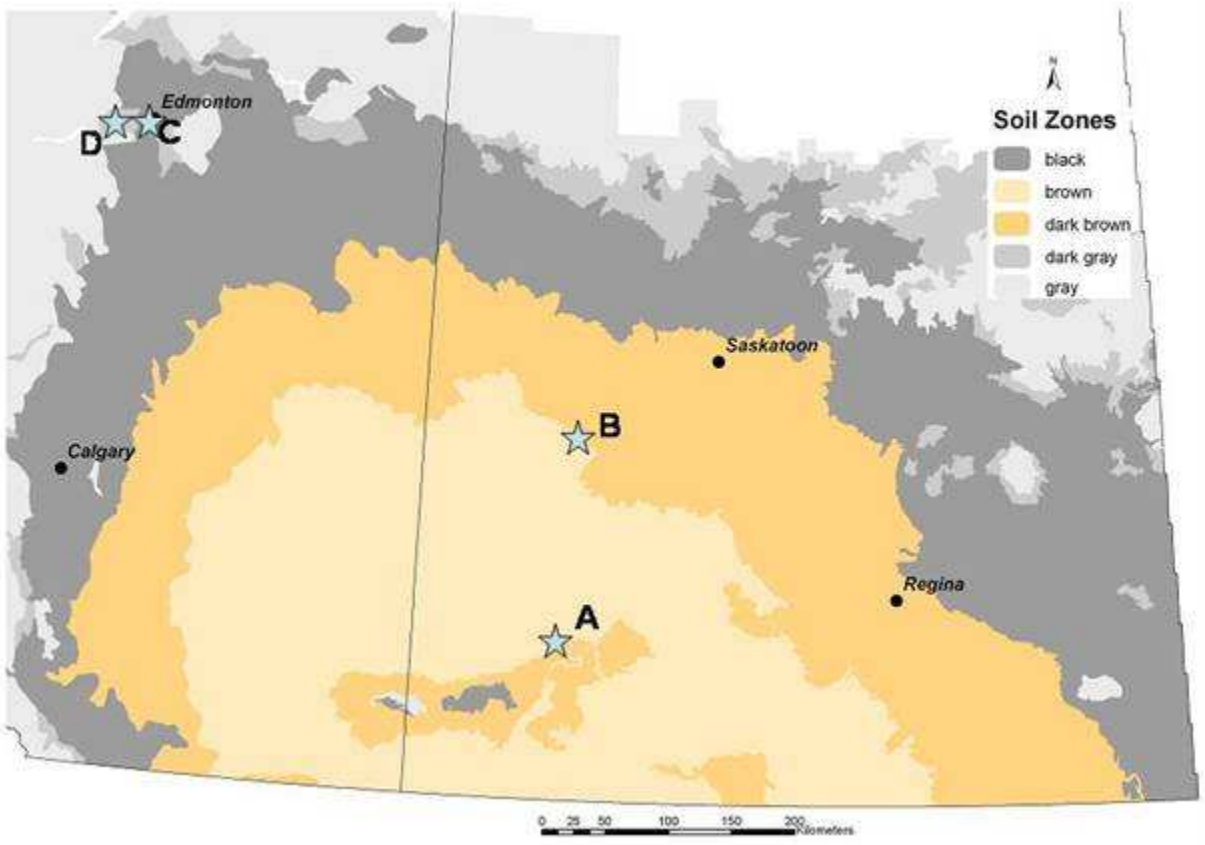

Figure A.1 Location map[1] of four long-term tillage trials used in the study of microbial dynamics in NT and CT soils at A) Swift Current, B) Scott, C) Ellerslie and D) Breton.

[1] Soil zone map provided by D. Cerkowniak, Saskatchewan Land Resource Unit.

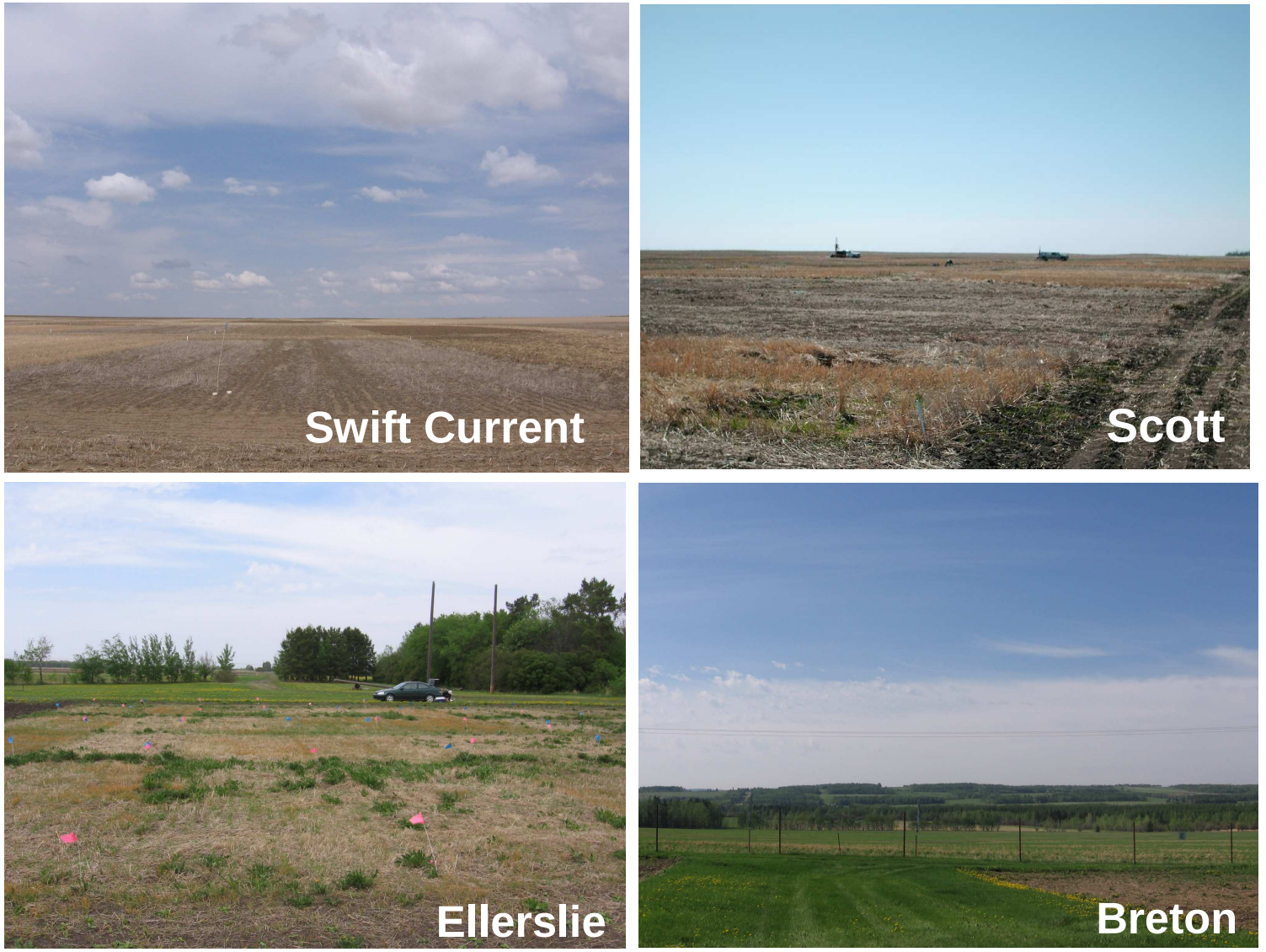

Figure A.2 Photographs of long-term field sites Swift Current, Scott, Ellerslie and Breton, taken prior to seeding in spring.

APPENDIX B

Microbial community analysis in NT and CT soils
Additional materials

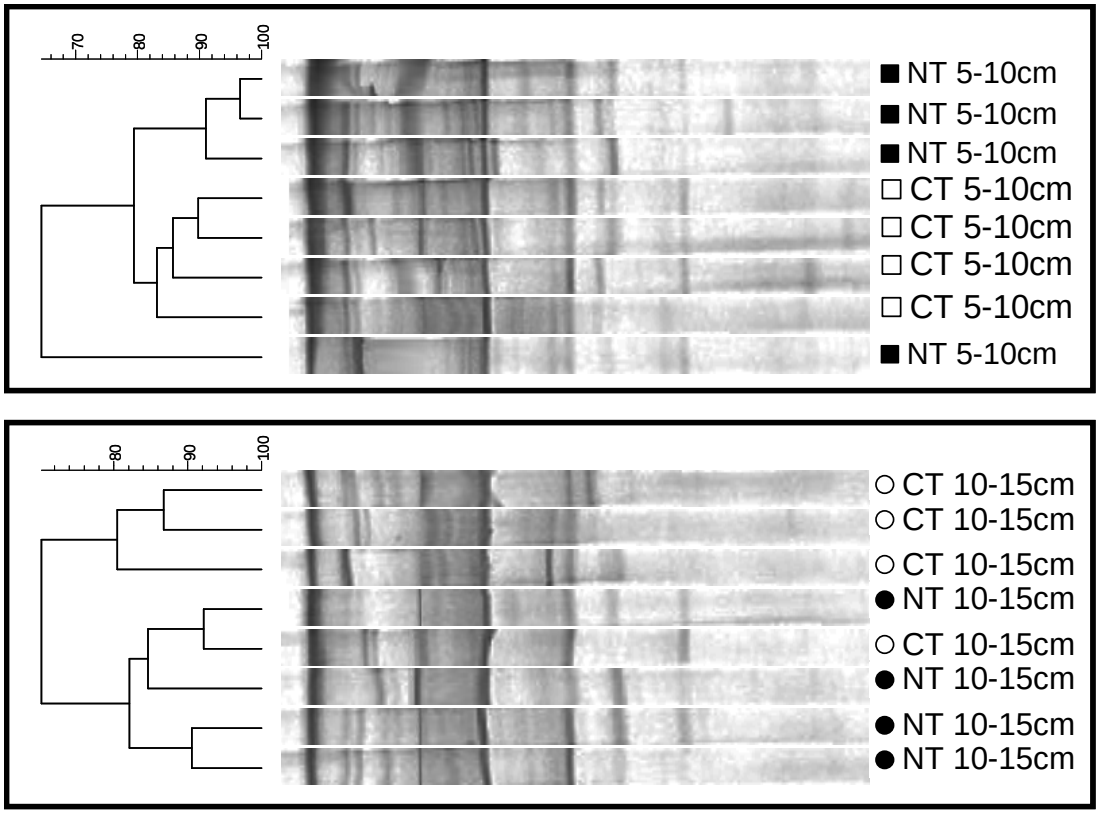

Figure B.1 Dendrograms of cluster analysis of no-till (NT) and conventionally-tilled (CT) 18S rDNA fingerprints for 5- to 10-cm and 10- to 15-cm depth increments at Swift Current.

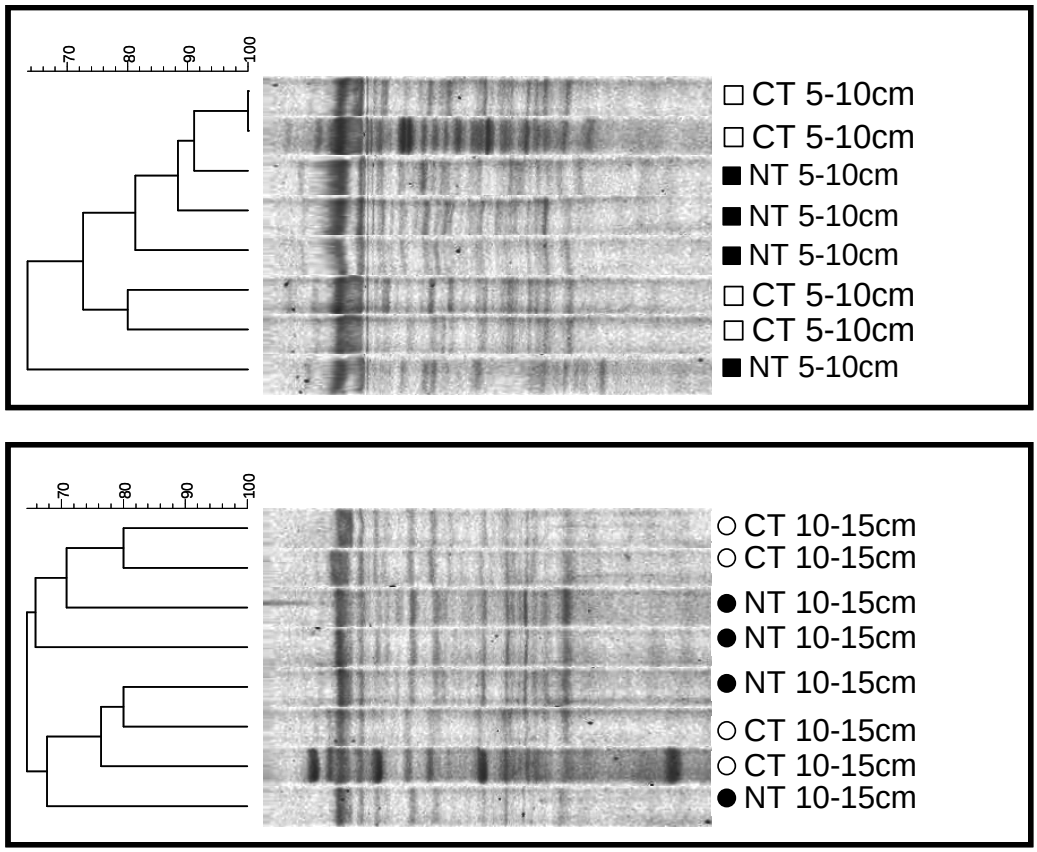

Figure B.2 Dendrograms of cluster analysis of no-till (NT) and conventionally-tilled (CT) 16S rDNA fingerprints for 5- to 10-cm and 10- to 15-cm depth increments at Swift Current.

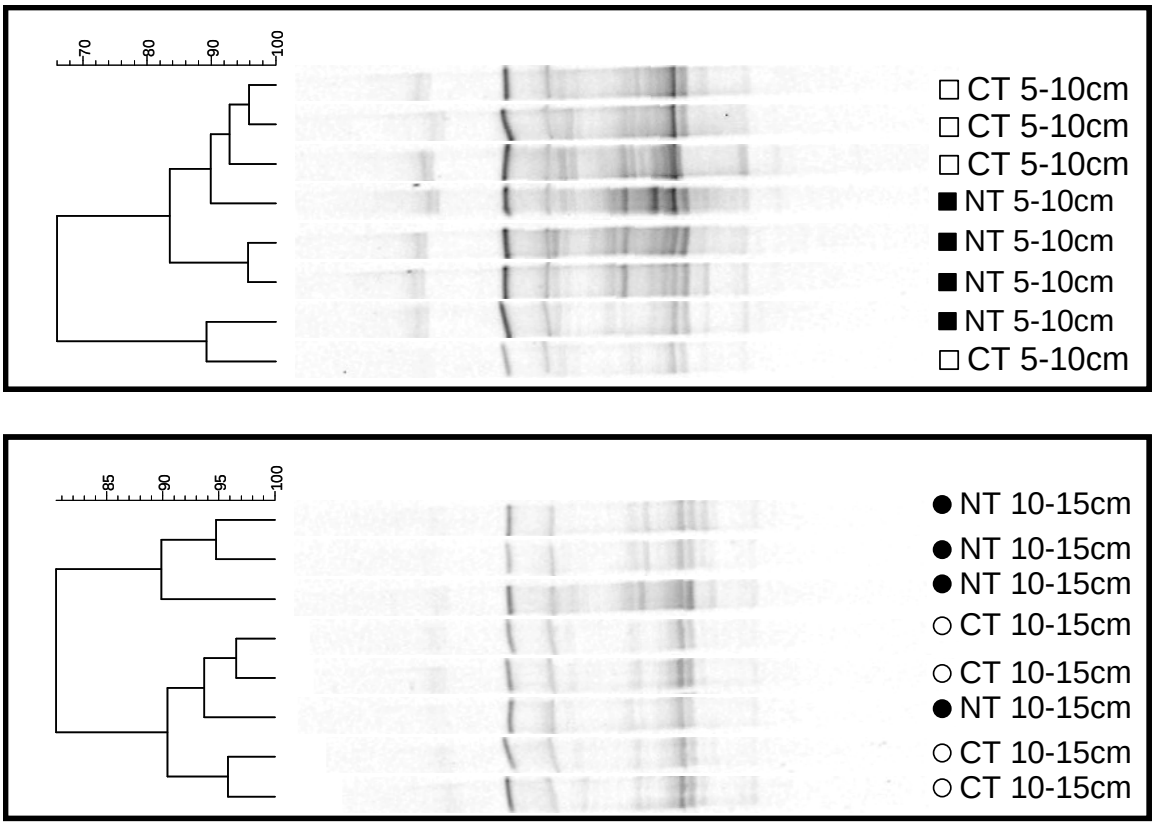

Figure B.3 Dendrograms of cluster analysis of no-till (NT) and conventionally-tilled (CT) 18S rDNA fingerprints for 5- to 10-cm and 10- to 15-cm depth increments at Scott.

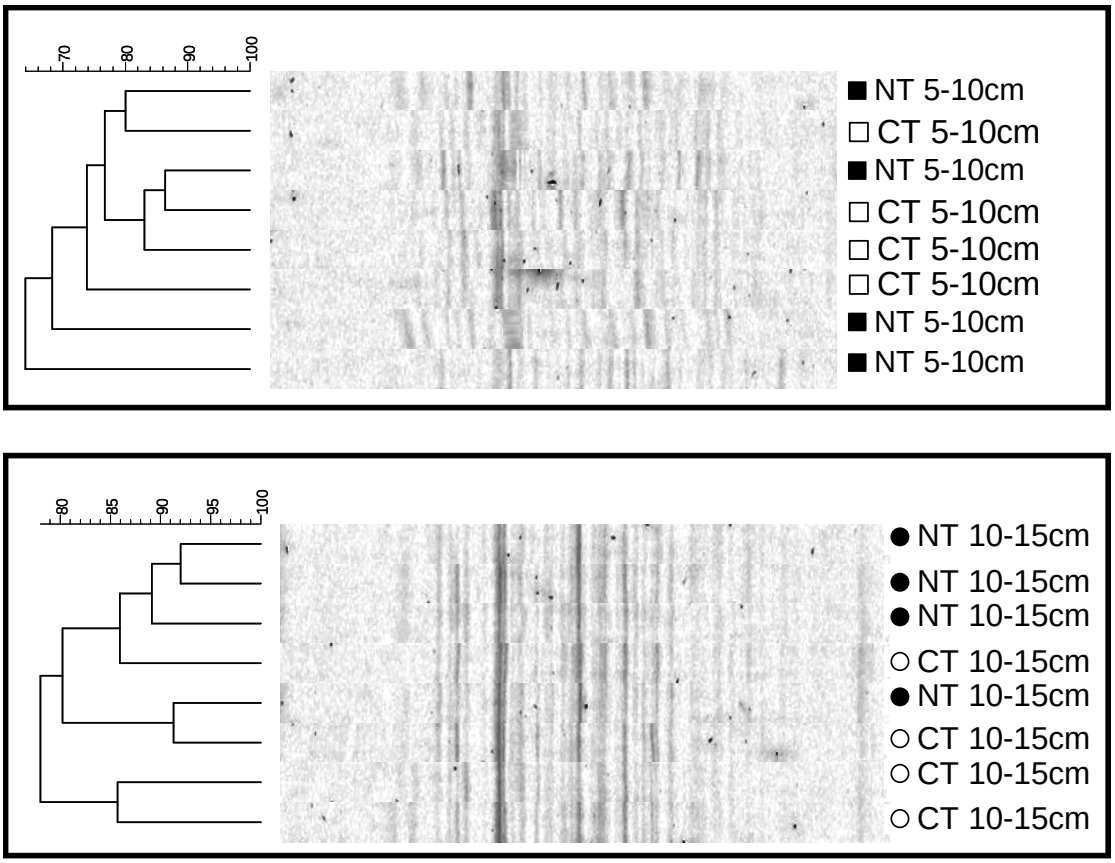

Figure B.4 Dendrograms of cluster analysis of no-till (NT) and conventionally-tilled (CT) 16S rDNA fingerprints for 5- to 10-cm and 10- to 15-cm depth increments at Scott.

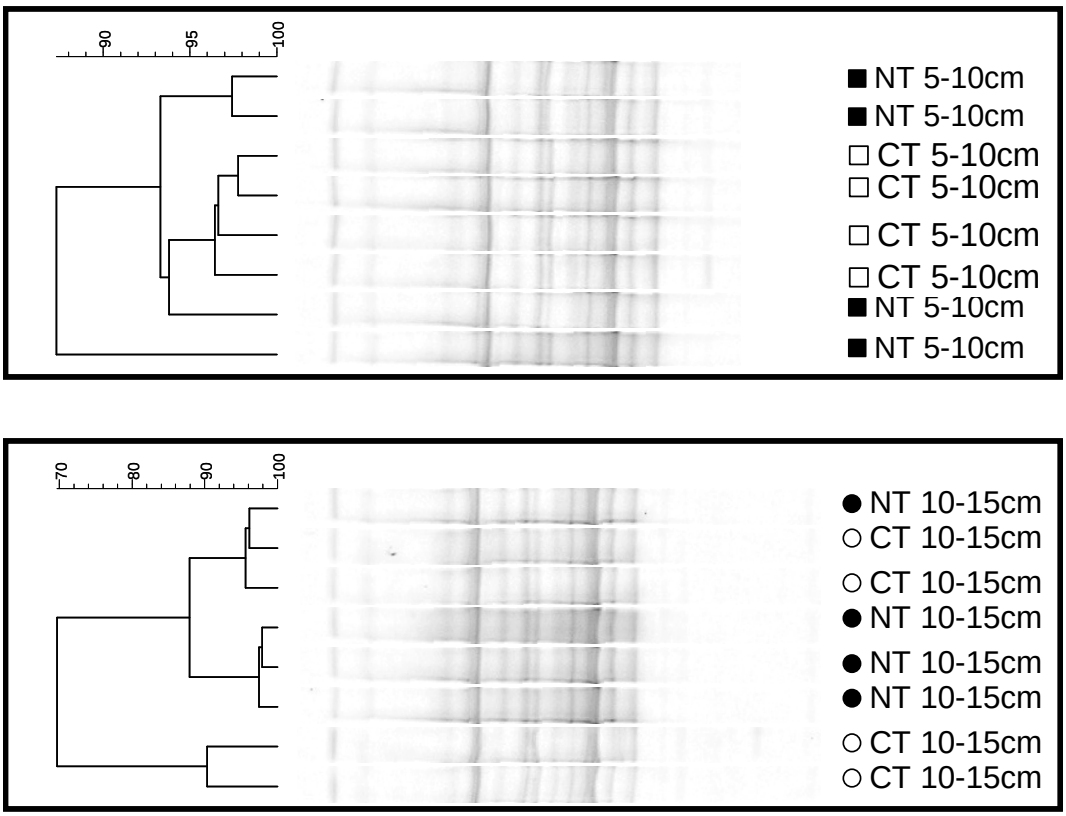

Figure B.5 Dendrograms of cluster analysis of no-till (NT) and conventionally-tilled (CT) 18S rDNA fingerprints for 5- to 10-cm and 10- to 15-cm depth increments at Ellerslie.

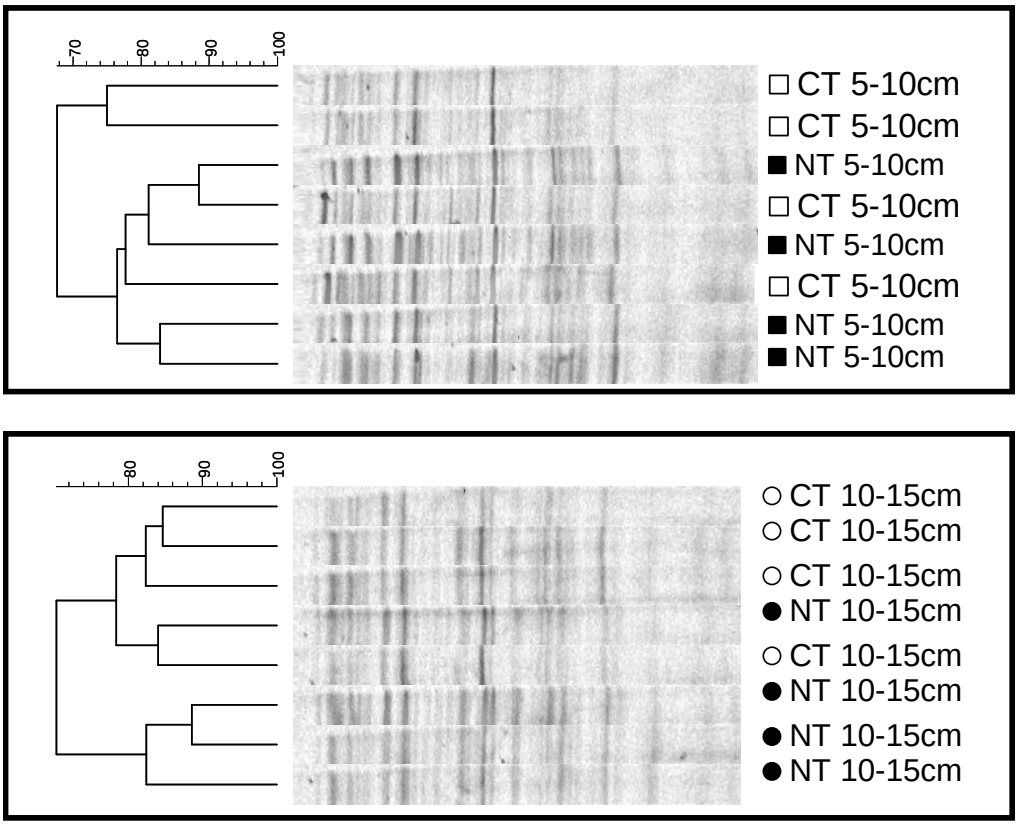

Figure B.6 Dendrograms of cluster analysis of no-till (NT) and conventionally-tilled (CT)16S rDNA fingerprints for 5- to 10-cm and 10- to 15-cm depth increments at Ellerslie.

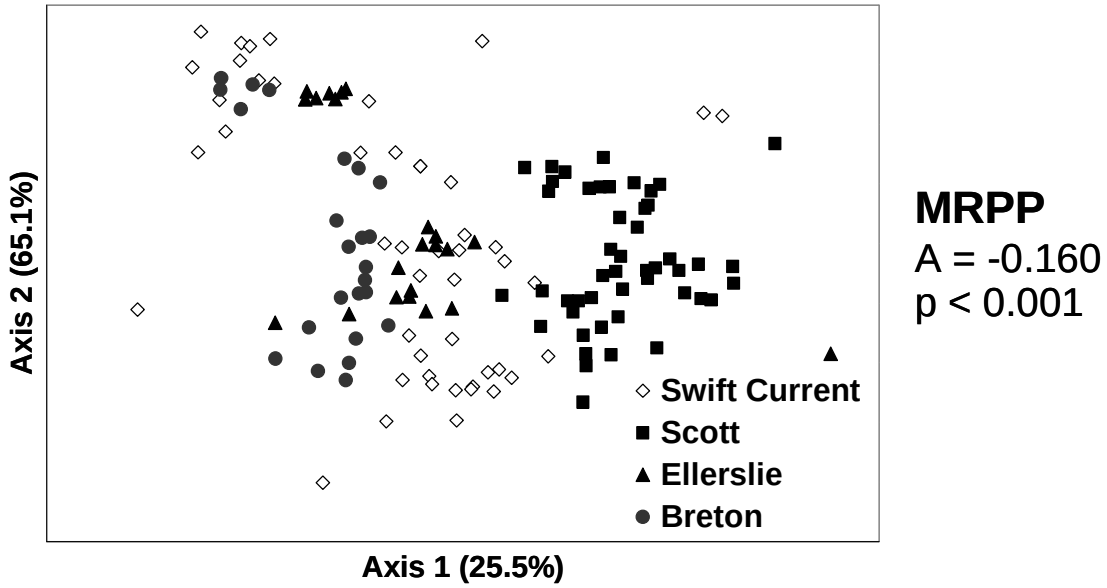

Figure B.7 Non-metric multidimensional scaling analysis (final stress = 14.1) and multiple response permutation procedure (MRPP) analysis of site effects on PLFA profiles (mol % data) from 2005 and 2006 field soils at four long-term tillage research sites.